

# **Novel Benzindoloazecines and Dibenzazecines - Synthesis and Affinities for the Dopamine Receptors**

Dissertation

zur Erlangen des akademischen Grades

Doctor rerum naturalium

(Dr. rer. nat.)

Vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät  
der Friedrich-Schiller-Universität Jena

von

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geboren am 17. Oktober 1978 in Alexandria

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Tag der öffentlichen Verteidigung: 5. Mai 2011

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## 1. Introduction

It was in the late 1950's after the discovery of dopamine's wide distribution in the brain<sup>1</sup>, that dopamine's function as a neurotransmitter and not as previously believed a mere precursor in the biosynthesis of epinephrine and norepinephrine was first recognized. Interest in dopamine grew after subsequent findings of its role in the pathogenesis of Parkinson's disease<sup>2</sup> and schizophrenia<sup>3</sup>.

Today, dopamine is known as a major neurotransmitter in the CNS, where it controls a myriad of physiological functions, including locomotion, behavior, emotion, cognition, learning and motivation in addition to endocrine secretion. Dysfunctions of the dopaminergic system have been linked with several neurological and psychiatric disorders in addition to Parkinson's disease and schizophrenia. These include depression<sup>4</sup>, Tourette's syndrome<sup>5</sup>, attention-deficit hyperactivity disorder (ADHD)<sup>6</sup> as well as drug and alcohol dependence<sup>7</sup>. Modulation of the dopamine transmission by targeting either the dopamine receptors or its transporter is hence a main strategy or central focus of research in the treatment of these disorders.

The search for new ligands acting at the different dopamine receptors is for several reasons still of major importance.

The lack of subtype selective ligands, especially those which are able to differentiate between D<sub>1</sub> and D<sub>5</sub> receptors, has been a major impediment in the way of clarifying the physiological role of the individual receptors. Most of the studies concerning the functions of the dopamine receptors have been carried out using genetically modified mice. Although valuable information has been obtained, the inconsistency of the results and the limitations of this strategy have proven that subtype selective ligands are indispensable as pharmacological tools to study both the physiological role and the possible clinical implication of the different dopamine receptor subtypes.

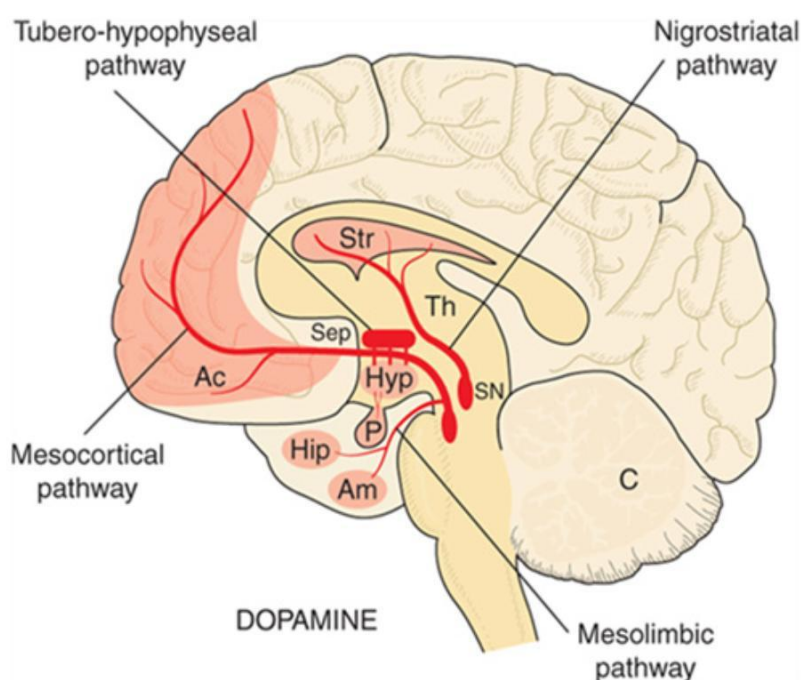
Subtype selective ligands would also be of major value in exploring the binding site of the dopamine receptors. They can help determine the structural, spatial and stereochemical features necessary for distinguishing between the different receptor subtypes. This will in turn help get further insights into the binding site of the receptor subtypes.

Dopamine receptor ligands have long been used in the therapy of neurological diseases, in particular of Parkinson's disease (agonists) and schizophrenia (antagonists). Despite the availability of numerous dopamine antagonists, used in the therapy of schizophrenia, limited therapeutic efficacy and/or undesired side-effects present major obstacles for a successful therapy. This has been partly resolved by the development of atypical antipsychotics. Several hypotheses have been developed in an attempt to explain the mechanism of action

behind the 'atypicality' of these compounds. To further clarify the possible mechanism, dopamine ligands with different receptor profiles (e.g. lower  $D_2$ , higher  $D_1$  or additional  $5HT_{2A}$  affinity) may become handy.

## Dopamine receptors

Dopamine receptors are mainly expressed in the brain in addition to some peripheral tissues. Eight different dopamine-mediated pathways have been identified in the brain, the following are of most physiological relevance<sup>8,9</sup>: (**Figure 1.1**)



**Figure 1.1: Dopamine pathways in the brain.** Ac: nucleus accumbens; Am: amygdala; C: cerebellum; Hip: hippocampus; Hyp: hypothalamus; P: pituitary gland; Sep: Septum; SN: substantia nigra; Str: corpus striatum; Th, thalamus. Figure taken from Rang & Dale's Pharmacology<sup>9</sup>.

- The nigrostriatal pathway: Almost 70% of the brain dopamine is localized in this system, which projects from the substantia nigra to the dorsal striatum (caudate nucleus and putamen). It is mainly involved in the control of motor function, hence the direct association with Parkinson's disease, which results from the degeneration of the dopaminergic neurons and the consequent depletion of dopamine in this region.
- The mesolimbic/mesocortical pathway: projecting from the ventral tegmental area (VTA) to respectively the limbic areas of the brain (nucleus accumbens, amygdala and ventral striatum) and the cortex. It has been associated with cognition, emotionality and motivation. Abnormalities of the dopamine transmission in this area have been linked with positive and negative symptoms of schizophrenia<sup>10</sup>. Drugs of

abuse are also believed to act via elevating the dopaminergic transmission in the nucleus accumbens (mesolimbic system).

- The tuberohypophyseal pathway: connecting the hypothalamus to the pituitary gland, and has an inhibitory control over prolactin secretion.

Peripherally, dopamine receptors are found in the cardiovascular system and the kidney where they affect vascular tone and sodium transport, respectively<sup>11</sup>.

Further variations in the dopaminergic functions result from the action at the different dopamine receptor subtypes. Dopamine receptors belong to the superfamily of G-protein coupled receptors (GPCRs). They have been subdivided into two families, as originally suggested by Keabian and Calne in 1979<sup>12</sup>, based on their interaction with adenylate cyclase (AC) and ligand binding. The D<sub>1</sub>-like family comprises D<sub>1</sub> and D<sub>5</sub> receptor subtypes, whereas the D<sub>2</sub>-like receptors encompass the D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> receptor subtypes. The two receptor families differ essentially in their structure, signal transduction pathways, tissue distribution and pharmacology.

### 1.1. Receptor structure

As already mentioned, dopamine receptors are members of the GPCRs, also known as seven-transmembrane domain receptors (7TM); with their characteristic structural features comprising an extracellular N-terminal, seven transmembrane  $\alpha$ -helices, connected by three extra- and three intracellular loops and finally an intracellular C-terminal.

The sequence identity between D<sub>1</sub>-like and D<sub>2</sub>-like receptors is only 44%, D<sub>1</sub>-like receptors have a shorter intracellular third loop than D<sub>2</sub>-like receptors, while their C-terminal is about seven times longer<sup>13</sup>.

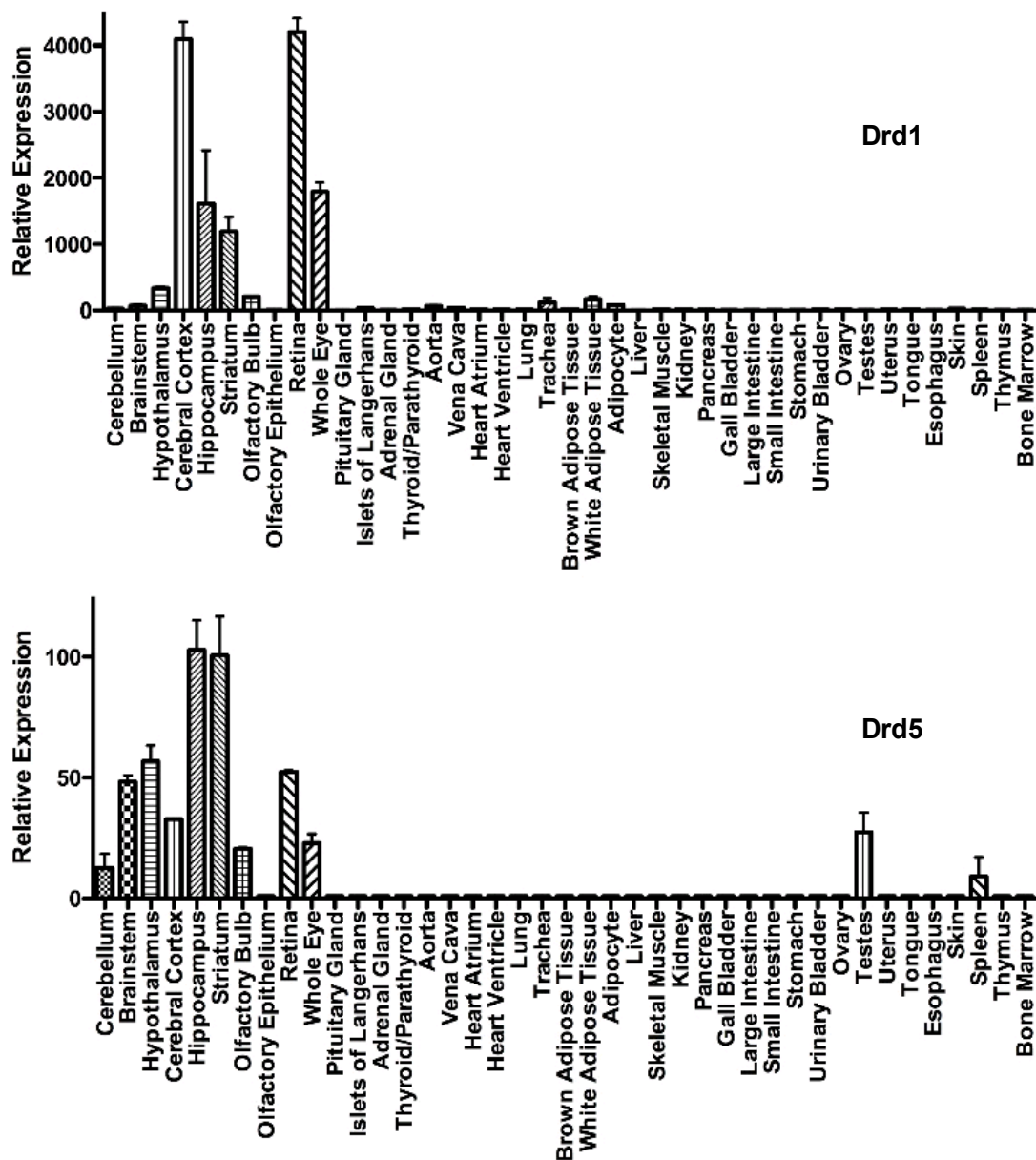
Both receptors of the D<sub>1</sub>-family are 80% identical in their primary sequence, D<sub>1</sub> containing 446 and D<sub>5</sub> 477 amino acids. Differences in the primary structure of both receptor subtypes are mainly observed in the third intracellular loop as well as in the external loop between the transmembrane domain (TM)4 and TM5<sup>8</sup>.

Within the D<sub>2</sub>-family, D<sub>2</sub> and D<sub>3</sub> share 75% homology, while D<sub>2</sub> and D<sub>4</sub> only have 53% conformity. The D<sub>2</sub> receptor subtype exists in two isoforms; D<sub>2S</sub> (D<sub>2short</sub>) and D<sub>2L</sub> (D<sub>2long</sub>), the latter containing 29 more amino acids in the third intracellular loop<sup>8</sup>. It has been postulated that D<sub>2S</sub>, which is mainly localized presynaptically, functions as an autoreceptor and regulates dopamine neurotransmission<sup>14</sup>.

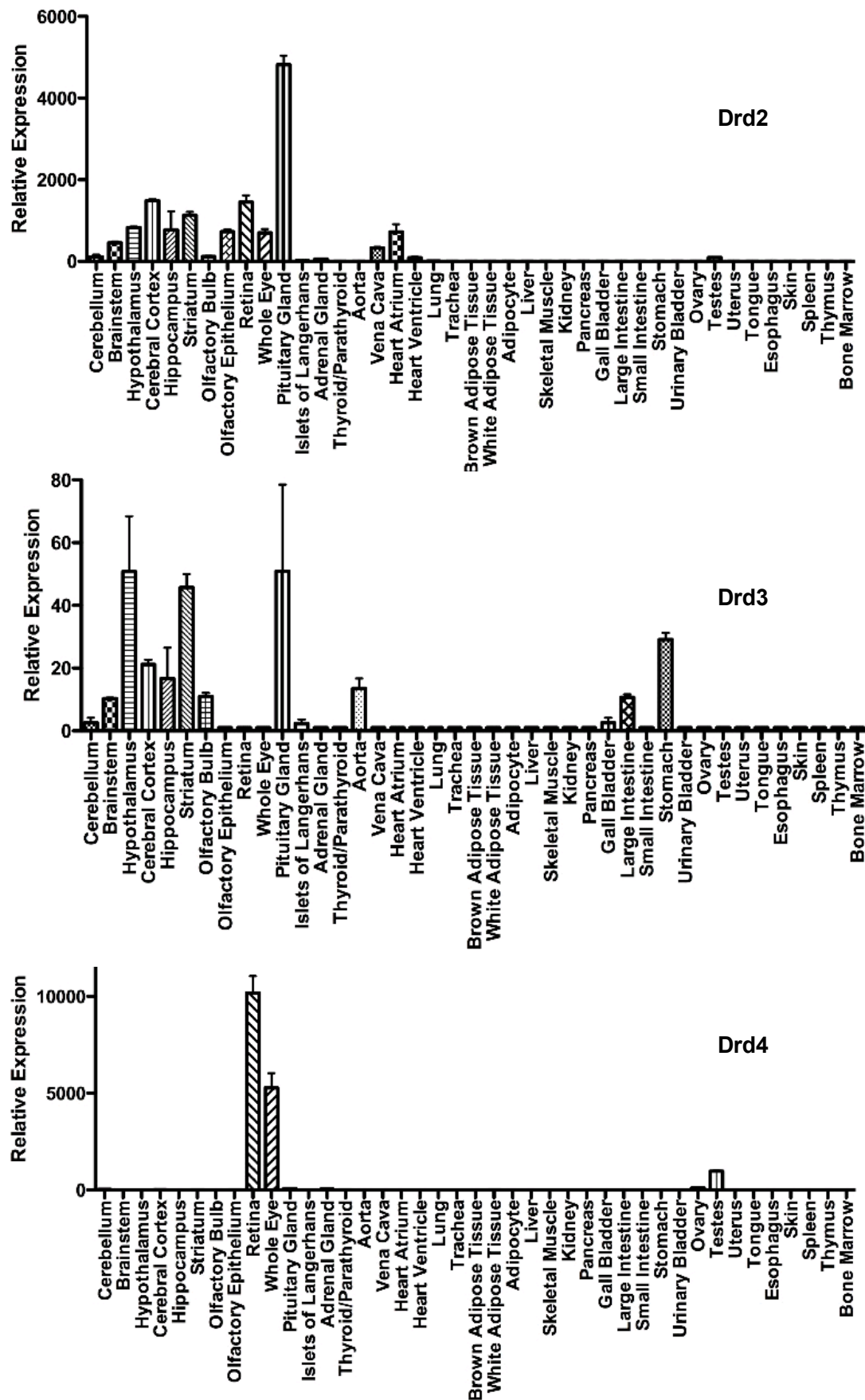
## 1.2. Tissue distribution

The different dopamine receptor subtypes show a more or less distinct anatomical localization in the brain tissues. **Figure 1.2** serves an illustrative overview over the relative distribution in the various tissues.

The D<sub>1</sub> receptor has the highest expression among all dopamine receptors; it is present with relatively high levels in all dopamine projection areas of the brain and has been mainly located in the putamen (dorsal striatum), nucleus accumbens (limbic area), cerebral cortex, hypothalamus and thalamus<sup>8;13;15</sup>. Conversely, the D<sub>5</sub> receptor is of low abundance and more localized in the hippocampus, prefrontal cortex, basal ganglia, thalamus and hypothalamus<sup>16</sup>. However, in the hippocampus the D<sub>5</sub> receptor is expressed in a higher level than the D<sub>1</sub> receptor<sup>16</sup>.







**Figure 1.2:** Tissue distribution of the mRNA of the different dopamine receptor subtypes. Graphs from PDSP<sup>17</sup> database.

The D<sub>2</sub> receptor subtype shows the second highest abundance in brain tissues; having a similar distribution as the D<sub>1</sub> receptor, except in the substantia nigra and VTA, where only D<sub>2</sub> has been localized. Both D<sub>3</sub> and D<sub>4</sub> receptors are less abundant; D<sub>4</sub> is the least expressed receptor subtype among all dopamine receptors. The D<sub>3</sub> receptor is largely localized in the limbic region and not expressed in the striatum. Similarly, the D<sub>4</sub> receptor is more expressed in the limbic region than in the striatum<sup>8;13;15</sup>.

### 1.3. Signal transduction

Stimulation of dopamine receptors, as all GPCRs, does not lead to a direct alteration of the membrane potential of neurons. Instead, a cascade of intracellular reactions and various second messengers are activated through specific G-proteins and possibly other proteins. Consequently, the final effect of dopamine receptor stimulation does not only depend on the receptor subtype, but also on the signal transduction components of the affected tissues.

The D<sub>1</sub>-like receptors (D<sub>1</sub> and D<sub>5</sub>) couple to the stimulatory G-proteins G<sub>s</sub> and G<sub>olf</sub>, activating adenylate cyclase to increase cyclic adenosine monophosphate (cAMP) levels. The signaling pathway includes the consequent activation of protein kinase A (PKA) and hence stimulation of PKA-catalyzed phosphorylation of many substrates. These substrates include two subtypes of the glutamate receptors, namely AMPA and NMDA receptors, in addition to GABA receptors. Regulation of several ion channels, including Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>-ion channels, also occurs by modulating the phosphorylation state of these channels or that of associated proteins<sup>18;19</sup>. Another important substrate of PKA is DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa), which upon phosphorylation inhibits protein phosphatase 1 and thus amplifies the response to PKA and to other protein kinases<sup>18</sup>.

D<sub>2</sub>-like receptors couple to the inhibitory G-proteins G<sub>i</sub> and G<sub>o</sub>. They modulate many signaling pathways in addition to adenylate cyclase, including phospholipases, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>-ion channels as well as NMDA, AMPA and GABA receptors<sup>19</sup>.

The transduction mechanism of dopamine receptors is more complex *in vivo*; dopamine receptors (as many GPCRs) have the potential to form homo- and heterodimers as well as -oligomers. Homodimers and -oligomers of D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> receptors besides heterodimers of several dopamine receptors with other receptors have been localized. Examples of the latter are: D<sub>1</sub> and adenosine A<sub>1</sub> receptors, D<sub>2</sub> or D<sub>3</sub> and adenosine A<sub>2</sub> receptors, and D<sub>1</sub> and NMDA receptors. Moreover, D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> receptors build D<sub>1</sub>/D<sub>2</sub>, D<sub>1</sub>/D<sub>3</sub> and D<sub>2</sub>/D<sub>3</sub> heterodimers. These heterodimers show distinct functional properties and binding profiles from the respective monomers<sup>20;21</sup>.

### 1.4. Functions of the dopamine receptors and their therapeutic implications

As already mentioned, dopamine receptors are involved in the control of many physiological functions in the CNS as well as in the periphery. The functions of the individual receptor subtypes are till the present not completely discerned. This is partly owing to the lack of selective ligands at some of the receptor subtypes, which can be utilized as pharmacological tools to clarify the specific roles of each subtype. Many studies have thus been based on knockout (KO) mice in an attempt to elucidate these distinct functions.

Subsequently, a brief description of the more relevant roles of the dopamine receptors will be given, with a focus on D<sub>1</sub>-family receptors, as they constitute the main target in this work.

#### 1.4.1. Control of locomotion and application in movement disorders

Dopamine receptors are key regulators of motor functions in the CNS and consequently play an elemental role in the pathogenesis of several movement disorders. Most importantly is their role in Parkinson's disease, where motor impairment is a direct result of the degeneration of the dopaminergic neurons in the striatum. Alleviation of the motor symptoms in Parkinson's disease is mainly achieved by the application of dopamine agonists. Dopamine agonists have found therapeutic use in other movement disorders, such as restless leg syndrome<sup>22</sup>. On the other hand, dopamine antagonists are applied to reduce tics in Tourette's syndrome<sup>23</sup> and chorea in Huntington's disease<sup>24</sup>.

The most therapeutically used dopamine agonists are more or less selective for the receptors of the D<sub>2</sub>-family. The role of the different receptor subtypes of the D<sub>2</sub> family is however not completely cleared<sup>25</sup>. While the role of the D<sub>2</sub> receptor subtype in the control of motor function has been well established; it has a stimulatory role on locomotion, while its blockade leads to the reduction of locomotor activity, the functions of both D<sub>3</sub> and D<sub>4</sub> in locomotion are not completely clear yet.

Within the D<sub>1</sub> family, D<sub>1</sub> as well as D<sub>5</sub> receptors have been both implicated in the control of motor behavior. There are some indications that D<sub>5</sub> acts synergistically with D<sub>1</sub> in the control of motor functions<sup>26</sup>. Although studies on the locomotor activity of D<sub>1</sub> KO mice showed conflicting results with respect to its exact role<sup>26;27</sup>, findings that D<sub>1</sub> agonists stimulate locomotor activity and possess antiparkinsonian potential<sup>28;29</sup>, while antagonists show an inhibitory effect on locomotion, are evidence of the involvement of the D<sub>1</sub> receptors in the control of motor functions<sup>30</sup>. For instance, the D<sub>1</sub> full agonist dinapsoline (**figure 1.9**) has been found to be promising as antiparkinsonian agent<sup>28;29</sup>, with comparable efficacy to D<sub>2</sub> agonists and lower potential to cause dyskinesia<sup>28</sup>.

### 1.4.2. Role in reward and reward-seeking behavior and clinical implication in drug abuse and addictive disorders

Brain dopamine has been identified as the central neurotransmitter in the reward system and has been associated with the processing of rewarding stimuli as well as reward-seeking behavior. Rewarding stimuli are translated into pleasurable experiences, which are liked, desired and pursued<sup>31</sup>. Natural rewarding stimuli such as food, drink and sex in addition to abnormal reward-seeking behavior including gambling, excessive eating and consumption of drugs of abuse and alcohol, all ultimately increase the dopamine levels in the nucleus accumbens, though by different mechanisms<sup>32</sup>. Although the connection of the dopaminergic neurotransmission with reward and accordingly addiction has long been established, the precise role of dopamine is still not completely understood. Dopamine receptors have been postulated to mediate the enjoyment of rewards (pleasurable effect, for example the euphoria associated with psychostimulants), the learned predictions of future reward (learning) as well as to motivate the active seeking of rewards (wanting)<sup>33</sup>.

Many studies have thus been concerned with investigating the role of the individual dopamine receptor subtypes in addiction and addictive disorders with respect to both the mediation of the reward-stimulating and reward-seeking effects.

Stimulation of the D<sub>1</sub> receptor subtype has been linked with ethanol<sup>34</sup>, food<sup>35</sup> and psychostimulant reward<sup>36;37</sup>. For instance, deletion of the D<sub>1</sub> receptor subtype in mice models resulted in the attenuation of the hyperactivity, commonly induced by psychostimulants, and in reduced alcohol consumption<sup>26</sup>. The role of the D<sub>5</sub> receptor is still ambiguous, it is believed that it participates in mediating at least the locomotor stimulant effect of cocaine<sup>38</sup>. These findings encouraged the investigation of the applicability of D<sub>1</sub> agonists and antagonists in the treatment of addiction and addictive behaviors.

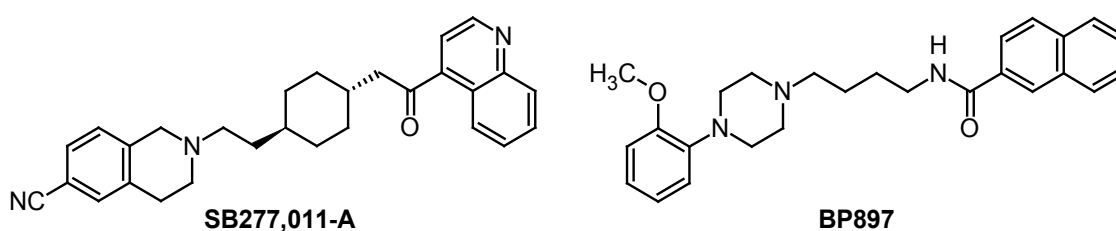
Although the characteristic locomotor stimulant effect of psychostimulants such as amphetamine and cocaine was reduced in D<sub>1</sub> KO mice<sup>37</sup>, conflicting results with respect to the effects of D<sub>1</sub> agonists and antagonists on the behavioral effects of cocaine were obtained. Some studies show that cocaine-seeking and self administration can be diminished by D<sub>1</sub> agonists<sup>39;40</sup>. In opposition, others argue that D<sub>1</sub> antagonists can lessen the incidence of relapse to cocaine self-administration<sup>41</sup> besides attenuating its euphoric effects<sup>42</sup>.

On the other hand, the ability of D<sub>1</sub> antagonists to attenuate alcohol reward and seeking behavior in mice is rather well established<sup>43;44</sup>, which is coherent with findings showing that alcohol-seeking was significantly reduced in D<sub>1</sub> KO mice<sup>45</sup>.

Some D<sub>1</sub> antagonists were inferred to be potentially useful for the treatment of addiction. For instance, the tetrahydroisoquinoline D<sub>1</sub> antagonist BTS73947 (see **figure 1.6**) was patented for the use in addictive disorders, as it was found to reduce the craving for addictive

substances as cocaine, amphetamine, nicotine, opiates, tobacco and alcohol as well as food cravings<sup>46</sup>. Other D<sub>1</sub> antagonists were also patented for the treatment of obesity and eating disorders<sup>47;48</sup>. Ecopipam (SCH39166, see **figure 1.5**) is another D<sub>1</sub> antagonist which has been proven effective in the treatment of obesity and has even reached phase III trials. Further studies were however discontinued, as administration of ecopipam was accompanied by unexpected psychiatric side effects including depression, anxiety, and suicidal thoughts<sup>49</sup>. Another D<sub>1</sub> antagonist which has reached phase II trials is NNC687 (currently under the name ADX10061, **figure 1.4**). It has been tested for the treatment of alcohol and nicotine dependence<sup>50</sup>, but the clinical trials were also discontinued.

D<sub>2</sub> and D<sub>3</sub> subtypes have been connected with reward and with the various responses to substance of abuse. The D<sub>3</sub> receptor has gained special attention in this respect, due to its concentration in the limbic area. Selective D<sub>3</sub> antagonists, including SB277,011-A and BP897 (**Figure 1.3**) were found to inhibit cocaine, alcohol and nicotine seeking behavior<sup>51;52</sup>. This gives hope for their possible clinical potential in the treatment of addiction.



**Figure 1.3:** Selective D<sub>3</sub> antagonists SB277,011-A and BP897.

### 1.4.3. Role in psychosis and schizophrenia

Schizophrenia is a chronic mental disorder affecting mainly the cognitive, emotional and behavioral state. The clinical picture of schizophrenia is characterized by the presence of multiple symptoms. Psychotic symptoms, manifested in the form of hallucinations and delusions, have been termed positive symptoms. Negative symptoms, including social withdrawal, apathy and anhedonia, accompanied by cognitive deficits such as disorganized thoughts, difficulty concentrating, difficulty completing tasks and memory problems are also typical for that disorder.

Early links of dopamine to schizophrenia originate from van Rossum's postulation that a hyperactivity of the dopaminergic system in some brain regions may underlie the development of this disorder<sup>3</sup>. This hypothesis, later known as the dopamine hypothesis of schizophrenia, was first supported by observations that amphetamine, which acts mainly by increasing dopamine levels, induces similar psychosis to schizophrenia. Later verifications came from studies showing that antipsychotics act as antagonists at the dopamine receptors, in particular at the D<sub>2</sub> receptors<sup>53;54</sup>. However, subsequent findings showed that schizophrenia is not just a result of dopaminergic hyperfunction. It has been proposed that positive symptoms are associated with hyperdopaminergia in the mesolimbic region, whereas negative and cognitive deficit symptoms are a result of hypodopaminergia in the mesocortical region, specifically in the prefrontal cortex (PFC)<sup>10</sup>.

D<sub>2</sub> receptors have for a long time been the main target of action of antipsychotics, compounds lacking D<sub>2</sub> affinities have no antipsychotic activity<sup>55</sup>. However, typical (first generation) antipsychotics, which act mainly by blocking the D<sub>2</sub> receptors, are usually only effective in alleviating the positive symptoms of schizophrenia, while negative symptoms and cognitive deficits respond poorly and may even worsen<sup>56</sup>. Moreover, hyperprolactinemia and Parkinsonian-like side effects, also known as extrapyramidal symptoms (EPS), resulting from dopamine receptor blockade in the striatum, are quite commonly encountered with the use of typical antipsychotics. Consequently, the sole role of D<sub>2</sub> receptors as therapeutic targets together with the notion that D<sub>2</sub> antagonism suffices for effective antipsychotic action has been reevaluated. This was encouraged by the fact that atypical (second generation) antipsychotics, which are characterized by their ability to improve both negative and cognitive symptoms with lower tendency to cause the common side effects, were found to possess different and varying receptor binding profiles. Hence, new potential target receptors and mechanisms of action have been studied.

Within the D<sub>2</sub>-family, D<sub>3</sub> receptors are also considered as attractive targets for antipsychotics. Antagonists at D<sub>3</sub> receptors are postulated to be effective against both positive and negative

symptoms and to have low potential to cause EPS, the latter fact is attributed to D<sub>3</sub>-receptors' limited distribution and their prevalence in the mesolimbic system<sup>51</sup>. In fact the atypical antipsychotic amisulpride has higher D<sub>3</sub> than D<sub>2</sub> affinity<sup>57</sup> and there is strong belief that mixed dopamine D<sub>3</sub>/D<sub>2</sub> receptor antagonists with preferential affinity to the D<sub>3</sub> receptor could be more effective as antipsychotics<sup>58</sup>. D<sub>4</sub> receptors were also considered as possible targets for antipsychotics. However, findings that D<sub>4</sub> selective antagonists are devoid of antipsychotic potential<sup>59</sup> indicate that D<sub>4</sub> antagonism does not suffice for antipsychotic action.

The potential antipsychotic effect of D<sub>1</sub> antagonists was also tested in patients suffering from schizophrenia. The results obtained were inconsistent. The D<sub>1</sub>/D<sub>5</sub> selective antagonist ecopipam (SCH39166; **figure 1.5**), displayed no antipsychotic effect<sup>60</sup>. However, another study showed that NNC687 (**figure 1.4**), also a D<sub>1</sub>/D<sub>5</sub> selective antagonist, possessed considerable antipsychotic potency, improved the negative symptoms and lacked the common extrapyramidal side effects (EPS)<sup>61</sup>. Its efficacy with respect to the negative symptoms was however disputed in other studies<sup>62</sup>. Declarative evidence of the effectiveness of D<sub>1</sub> antagonists in the treatment of schizophrenia is hitherto not available.

An opposite approach has been adopted by other studies. This has been based on results showing that D<sub>1</sub> receptors are involved in the control of cognition and working memory<sup>63</sup>. Dopamine neurotransmission in the PFC plays a major role in cognitive function<sup>64</sup>; in particular D<sub>1</sub> receptors in this region are critical in mediating cognitive and working memory performance<sup>65,66</sup>. This is substantiated by several findings. The D<sub>1</sub> receptors are highly abundant in the PFC<sup>67,68</sup>; the D<sub>1</sub>-family are an order of magnitude more abundant in the PFC than the D<sub>2</sub>-family receptors. Moreover D<sub>1</sub> antagonists were found to have an impairing effect on working memory and cognition<sup>69</sup> while D<sub>1</sub> agonists showed an enhancing effect<sup>70</sup>. This effect is yet not so simple; the relationship between dopamine transmission and working memory has been described as an "inverted U-shaped" one. A normal range of D<sub>1</sub> stimulation is required to maintain the integrity of working memory; excessive blockade and elevation of dopamine in the PFC both result in the deterioration of working memory<sup>71</sup>. Consequently, several studies have been advocating the potential benefits of using D<sub>1</sub> agonists for relieving the deficit symptoms of schizophrenia<sup>72-74</sup>. This has been based on the aforementioned findings, that D<sub>1</sub> stimulation in the PFC enhances cognitive performance and that intermittent doses of dopamine agonists show a long lasting cognitive enhancement<sup>70</sup>. In fact, l-stepholidine (**figure 1.8**), a naturally occurring alkaloid with a unique binding profile as a D<sub>1</sub> agonist and D<sub>2</sub> antagonist, showed promising results as potential antipsychotic<sup>74,75</sup>.

### 1.5. Dopamine receptor ligands

Dopamine receptor ligands are structurally diverse, nevertheless the majority shares some common structural features, which are considered necessary for binding to the receptor. Studies on ligand binding at catecholamine receptors in general and at modeled dopamine receptors (specifically D<sub>2</sub>) revealed some main structural features, essential for binding to the dopamine receptors and predicted key interaction sites in the receptor binding pocket. The binding pocket of all catecholamine receptors comprises an Asp residue at position 3.32, which builds a salt bridge to the protonated basic *N* of the ligand and is surrounded on either sides by two hydrophobic cavities<sup>76;77</sup>. Dopamine agonists are believed to activate the receptors by further interaction of their two catecholic hydroxyl groups with two Ser residues in TM5 by H-bond formation. Antagonists, on the other hand, only form weak interactions with one of the Ser residues in TM5<sup>77;78</sup>. This rule is however not unequivocal; some dopamine agonists, such as benzergolines, lack this catecholic function<sup>79</sup>. Other crucial interaction sites are the hydrophobic cavities on both sides of Asp3.32, which can accommodate the aromatic moieties of the ligands<sup>80</sup>. This model explains why the structure of many receptor ligands encompasses a central basic nitrogen surrounded by two aromatic moieties. To serve the goal of this work, this part will mainly focus on classes of dopamine receptor ligands which bear this structural characteristic.

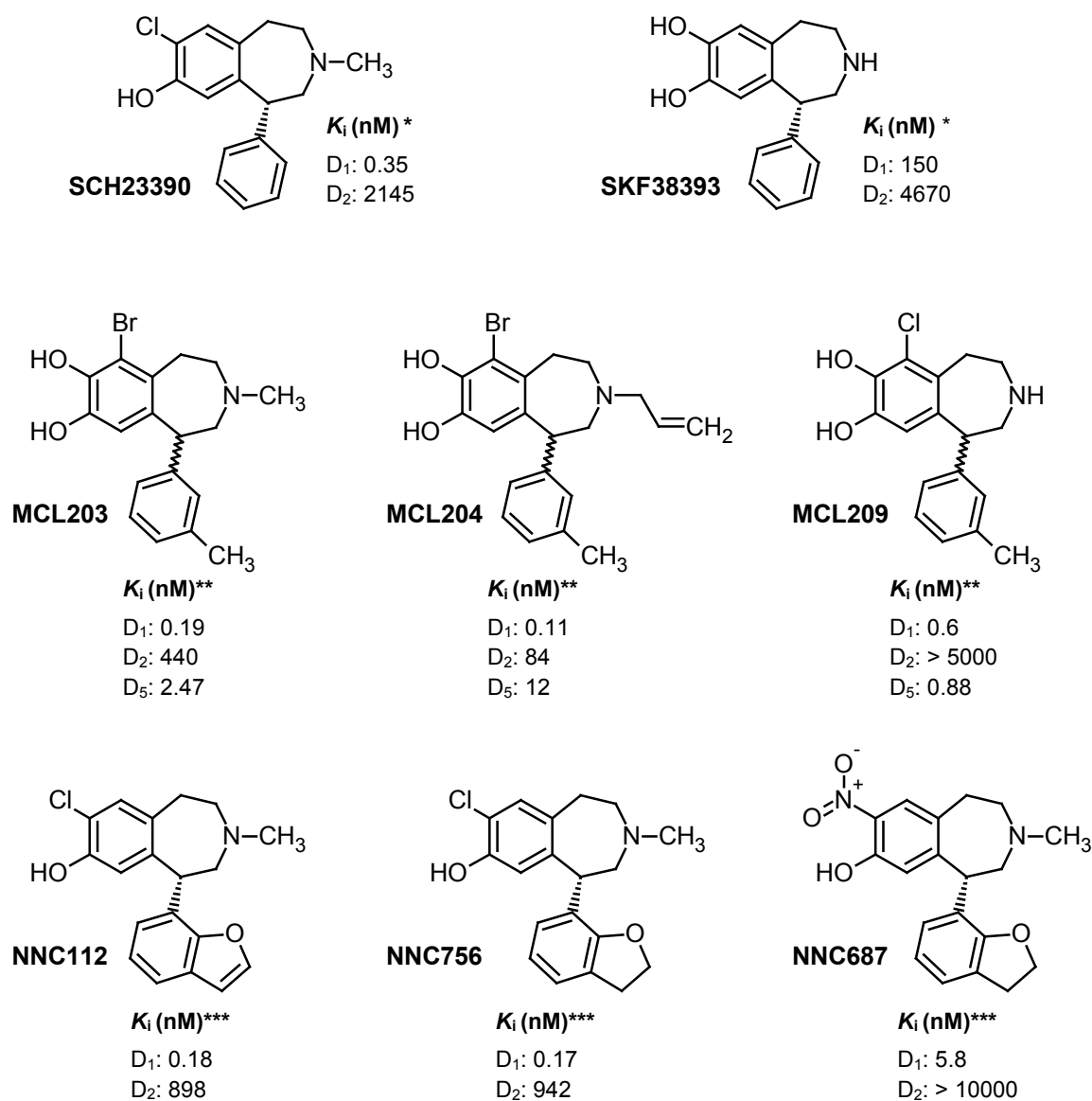
#### 1.5.1. Phenylbenzazepines

This class represents one of the most important classes of D<sub>1</sub> selective agonists and antagonists, which have made a major breakthrough in the pharmacology of dopamine receptors. (**Figure 1.4**)

The prototype SCH23390 is the first discovered D<sub>1</sub> antagonist, showing high preference for D<sub>1</sub>- with respect to D<sub>2</sub>-like receptors<sup>81;82</sup>. All antagonists within this class bear a halogen substitution at position 7 (SCH series), whereas compounds having a catecholic function, i.e. 7,8-dihydroxy substitution, are potent D<sub>1</sub>-agonists (SKF series)<sup>83</sup>. Several structural modifications have been introduced into the structure of 1-phenylbenzazepines. Neumeyer et al. introduced minor changes into the structure of the dopamine agonists of the SKF series by changing the substituent at the *N* and at C6 of the benzazepine moiety as well as at the phenyl ring<sup>84</sup>. Most of the obtained compounds showed more or less high affinities for the D<sub>1</sub>-like family. The most active were MCL203, MCL206 and MCL204, showing subnanomolar to nanomolar affinities for D<sub>1</sub>-like receptors, and the latter showing 100 fold D<sub>1</sub> over D<sub>5</sub> selectivity. The D<sub>1</sub>/D<sub>2</sub> selectivity was retained for all compounds, nevertheless the degree of



selectivity varied greatly, since the affinities for D<sub>2</sub> receptors were also divergent, ranging from 20 to >5000 nM<sup>84</sup>.

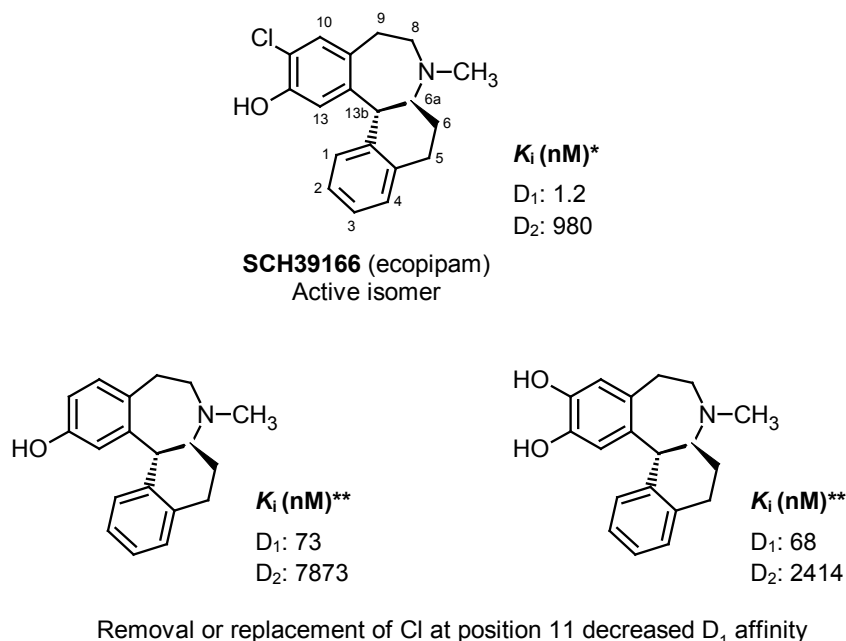


**Figure 1.4:** Structures of 1-phenylbenzazepines and their substituted derivatives. \*Values cited from PDSP<sup>17</sup> database; \*\*Values cited from reference<sup>84</sup>; \*\*\*Values cited from reference<sup>85</sup>.

Several structural modifications have also been carried out on the prototype D<sub>1</sub> selective antagonist SCH23390. The phenyl ring was substituted with a benzofuran and dihydrobenzofuran moiety, yielding highly selective D<sub>1</sub>-like antagonists, namely NNC112, NNC687 and NNC756. (**Figure 1.4**) These were found to be potent dopamine receptor, similarly showing a pronounced D<sub>1</sub>/D<sub>2</sub> selectivity<sup>85</sup>.

Further structural variations on SCH23390 included rigidifying the structure and reducing the flexibility of the phenyl ring<sup>86</sup>. SCH39166 (**figure 1.5**), also known as ecopipam, is the most prominent representative of this series. It displayed nanomolar affinities for D<sub>1</sub>-like receptors,

though weaker compared with SCH23390, and a high selectivity over D<sub>2</sub> receptors. Several interesting results were obtained from this study. Out of the four possible isomers of SCH39166 the (-)-trans isomer (6a*S*, 13b*R*) showed the highest affinities, which proves that the *R* orientation of 1-phenylbenzazepines in general is crucial for high affinities. A further interesting finding was that the replacement of the Cl at position 11 with hydrogen, hydroxyl or methoxy group significantly reduced the affinities for the D<sub>1</sub> receptor<sup>86</sup>. (**Figure 1.5**)



**Figure 1.5:** Conformationally restricted benzazepines. \*Values cited from reference<sup>87</sup>; \*\*Values cited from reference<sup>86</sup>.

### 1.5.2. Tetrahydroisoquinoline derivatives

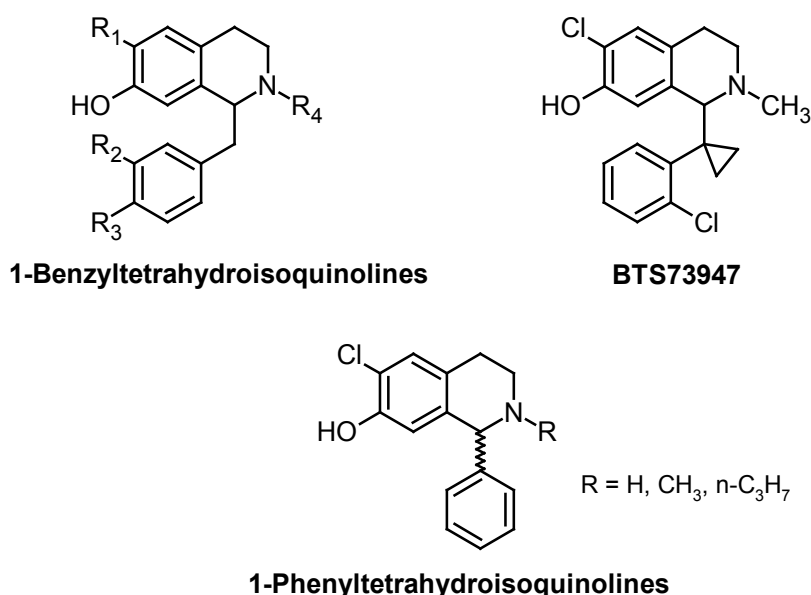
Tetrahydroisoquinoline ring is one of the most frequently encountered component in the structure of dopaminergic agents. Numerous dopamine ligands contain a tetrahydroisoquinoline moiety, either substituted with a benzyl/phenyl group or incorporated in a tetracyclic scaffold, with divergent annulation patterns.

#### 1.5.2.1. 1-Benzyl/1-phenyl tetrahydroisoquinolines

A large number of both naturally occurring and synthetic 1-benzyltetrahydroisoquinolines have been found to exert considerable affinities for dopamine receptors, though mostly only in the micromolar range. Here again, different substitution patterns were accompanied by different affinities and selectivity profiles. Kozlik et al. patented a series of 1-benzyltetrahydroisoquinolines substituted at the methylene bridge between the phenyl and the isoquinoline ring with bulky groups, including cyclopropyl, cyclobutyl, cyclopentyl and

cyclohexyl rings. These compounds showed high  $D_1/D_2$  selectivity with nanomolar affinities for  $D_1$  receptors. The most interesting compound in that series is BTS73957, which proved to be a potent and selective  $D_1$  antagonist<sup>88</sup>. (**Figure 1.6**)

On the other hand, several 1-phenyltetrahydroisoquinolines were synthesized in analogy to the  $D_1$  antagonist SCH23390. They all showed antagonistic activities at the dopamine receptors and a marked  $D_1/D_2$  selectivity. Contrary to phenylbenzazepines, the *S*-enantiomers were significantly more active than the *R*-enantiomers. The *N*-methyl substituted 1-phenylbenzazepine displayed the highest affinity at  $D_1$ -like receptors (6.6 nM) as well as the highest selectivity for  $D_1$  over  $D_2$  receptors. Increasing the length of the *N*-alkyl substituent resulted in decreased affinities<sup>89</sup>. (**Figure 1.6**)



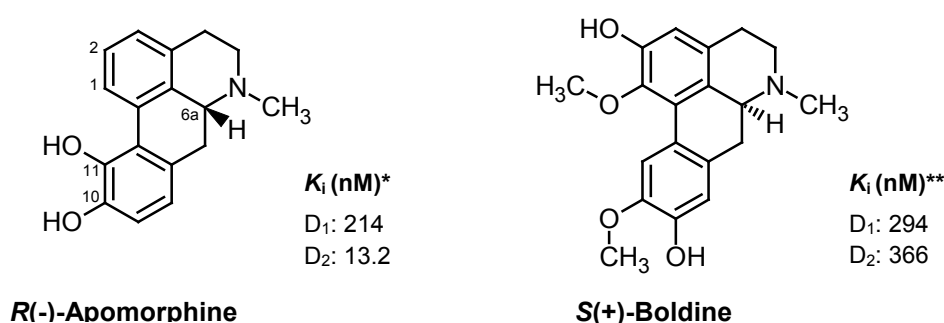
**Figure 1.6:** 1-Benzyl- and 1-phenyltetrahydroisoquinolines.

#### 1.5.2.2. Aporphines

The well-known dopamine agonist *R*(-)-apomorphine (**figure 1.7**), which is used in the treatment of Parkinson's disease and erectile dysfunction, is the most prominent member of this class of dopaminergic agents. It displays high affinities for all dopamine receptors, and has a rather interesting binding profile; displaying the highest affinity for  $D_4$  followed by  $D_5$  receptor, with markedly lower affinities for the  $D_1$  receptor<sup>90</sup>. Numerous structural variations have been introduced into the structure of apomorphine. Derivatives bearing only one hydroxyl substituent at position 11 were found to possess antagonistic rather than agonistic properties<sup>91</sup>, which is in line with the aforementioned speculations that a catecholic function is favored for receptor activation. Interesting was also that the nature of the *N*-alkyl substituent had a major impact on both the affinity and the selectivity of the compounds<sup>92-94</sup>; an *N*-propyl

substituent was found to be preferred by D<sub>2</sub> receptors, while *N*-methyl substitution was favored by D<sub>1</sub> receptors<sup>92;94</sup>. Moreover, while the *R*-enantiomers generally showed greater affinities than the *S*-enantiomers, the latter have been suggested to exhibit antagonistic rather than agonistic effects in behavioral studies<sup>95;96</sup>. For instance, *S*(+)-apomorphine has been suggested to possess dopamine antagonistic properties<sup>97</sup>.

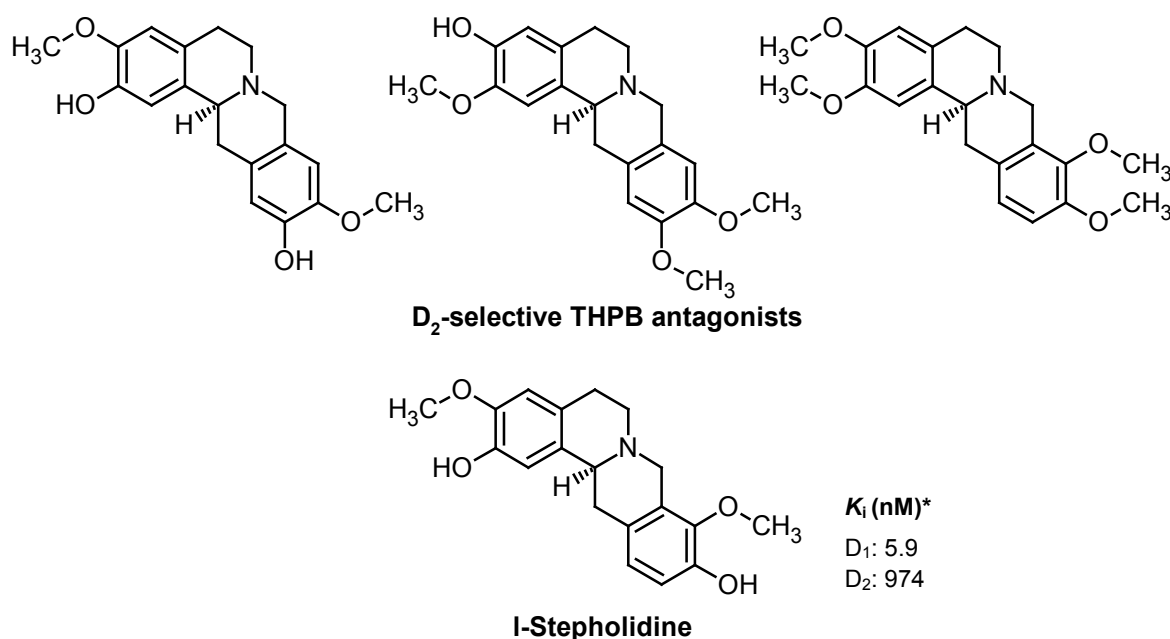
Similarly, the naturally occurring alkaloid *S*(+)-boldine, bearing an *S*-configuration at C6a, displayed dopamine antagonistic properties at the dopamine receptors. Halogenation of this antagonist at position 3 increased the affinities as well as the D<sub>1</sub>/D<sub>2</sub> selectivity. The 3-iodo derivative showed higher D<sub>1</sub> affinity than the 3-chloro and 3-bromo counterparts (*K<sub>i</sub>* = 2 nM, 60 nM and 152 nM, respectively)<sup>98;99</sup>.



**Figure 1.7:** Aporphines. \*Values cited from reference<sup>93</sup>; \*\*Values cited from reference<sup>100</sup>.

### 1.5.2.3. Tetrahydroprotoberberines (THPBs)

Several members of this class of naturally occurring alkaloids were found to act as antagonists at the dopamine receptors with a D<sub>2</sub>/D<sub>1</sub> selectivity profile<sup>99;101</sup>. (**Figure 1.8**)

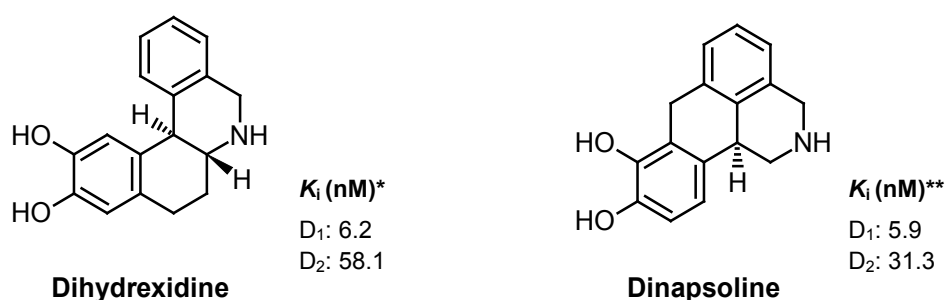


**Figure 1.8:** Tetrahydroprotoberberines. \*Values taken from PDSP<sup>17</sup> database.

Stepholidine (**figure 1.8**) is a unique member of this class. Not only does it show increased  $D_1$  affinities, but it also possesses a rare activity profile as a dual  $D_1$  agonist and  $D_2$  antagonist, which renders it attractive as potential antipsychotic agent<sup>74;102</sup>.

#### 1.5.2.4. Dihydraxidine and dinapsoline derivatives

Dihydraxidine (**figure 1.9**) is the first potent  $D_1$  full agonist, showing an intrinsic efficacy comparable to dopamine itself<sup>83</sup>. Similar to apomorphine, dihydraxidine has a conformationally rigid structure; it could be regarded as a rigid analogue of 4-phenyltetrahydroisoquinoline<sup>103</sup>.

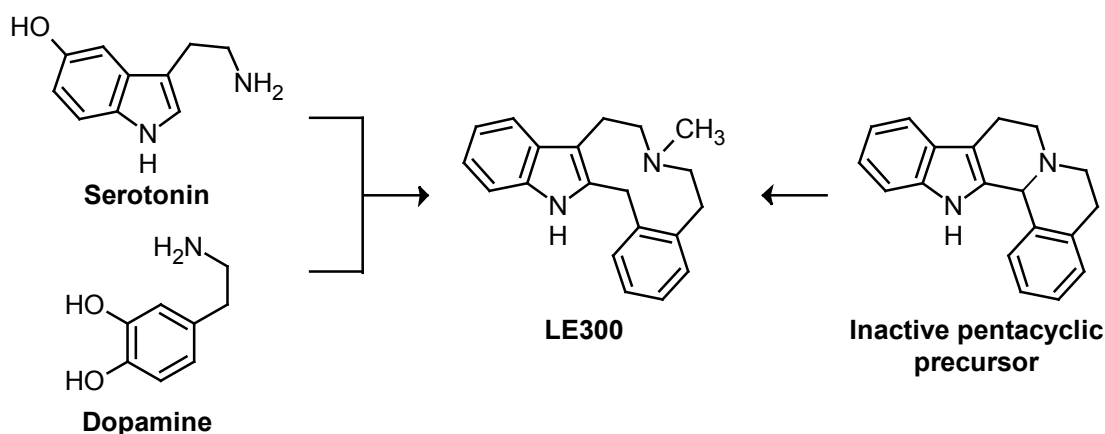


**Figure 1.9:** Structures of dihydraxidine and dinapsoline. \* Values taken from reference<sup>103</sup>, \*\*Values taken from reference<sup>104</sup>.

Comparable to previously mentioned dopamine ligands, *N*-methyl substitution of dihydraxidine resulted in the loss of  $D_1$  selectivity, while in the *N*-propyl derivative the affinity for  $D_2$  was higher than for  $D_1$ . Dinapsoline (**figure 1.9**) is another conformationally rigid analog of 4-phenyltetrahydroisoquinoline, which similar to dihydraxidine showed potent  $D_1$  full agonistic properties<sup>104</sup>.

### 1.5.3. Indolobenzazecines and Dibenzazecines

Indolo[3,2-*f*][3]benzazecines and dibenz[*d,g*]azecines, which have been developed in our research group, present a structurally novel class of dopamine receptor antagonists with interesting pharmacological profiles. The scaffold of the prototype LE300 has been designed to incorporate the structures of both dopamine and serotonin in a relatively flexible backbone. (**Figure 1.10**) The more rigid pentacyclic precursor of LE300 (**figure 1.10**) was found to be devoid of activity, which indicates that a moderate amount of flexibility is crucial for the dopamine affinities of this class of compounds<sup>105</sup>.



**Figure 1.10:** LE300 encompasses the structures of dopamine and serotonin in its scaffold.

Various modifications have been introduced into the structure of LE300 (**figure 1.11**), with the goal of drawing the SAR for this class of dopamine antagonists. The results obtained could be summed up as follows:

- Removal of the indole or the benzene ring as well as substitution of the annulated benzene ring with a phenyl group abolished the activities<sup>106</sup>.
- Replacement of the indole with other aromatic moieties was well tolerated; some dibenzazecine analogs were as active as the lead LE300<sup>106</sup>. Dibenzazecines have since been considered as another class of potent dopamine antagonists. These will be discussed later.
- Methyl substitution of the central alicyclic *N* was most favored; derivatives with longer alkyl or aralkyl chains as well as *NH* unsubstituted ones all possessed significantly lower affinities<sup>106</sup>.
- Methoxylation and hydroxylation of the indole ring increased the affinities for the D<sub>1</sub>-family<sup>107</sup>.
- Ring expansion to an 11-ring had different effects. Derivatives, where the tryptamine structure is maintained and the ring is elongated from the benzene side (i.e.

phenylpropyl moiety), possessed similar or increased affinities at some dopamine receptors as the lead LE300. Conversely, expanding the central ring from the indole side significantly attenuated the activity<sup>108</sup>.

- Contraction of the central alicycle to a 9-membered ring resulted in loss of affinities<sup>106</sup>.

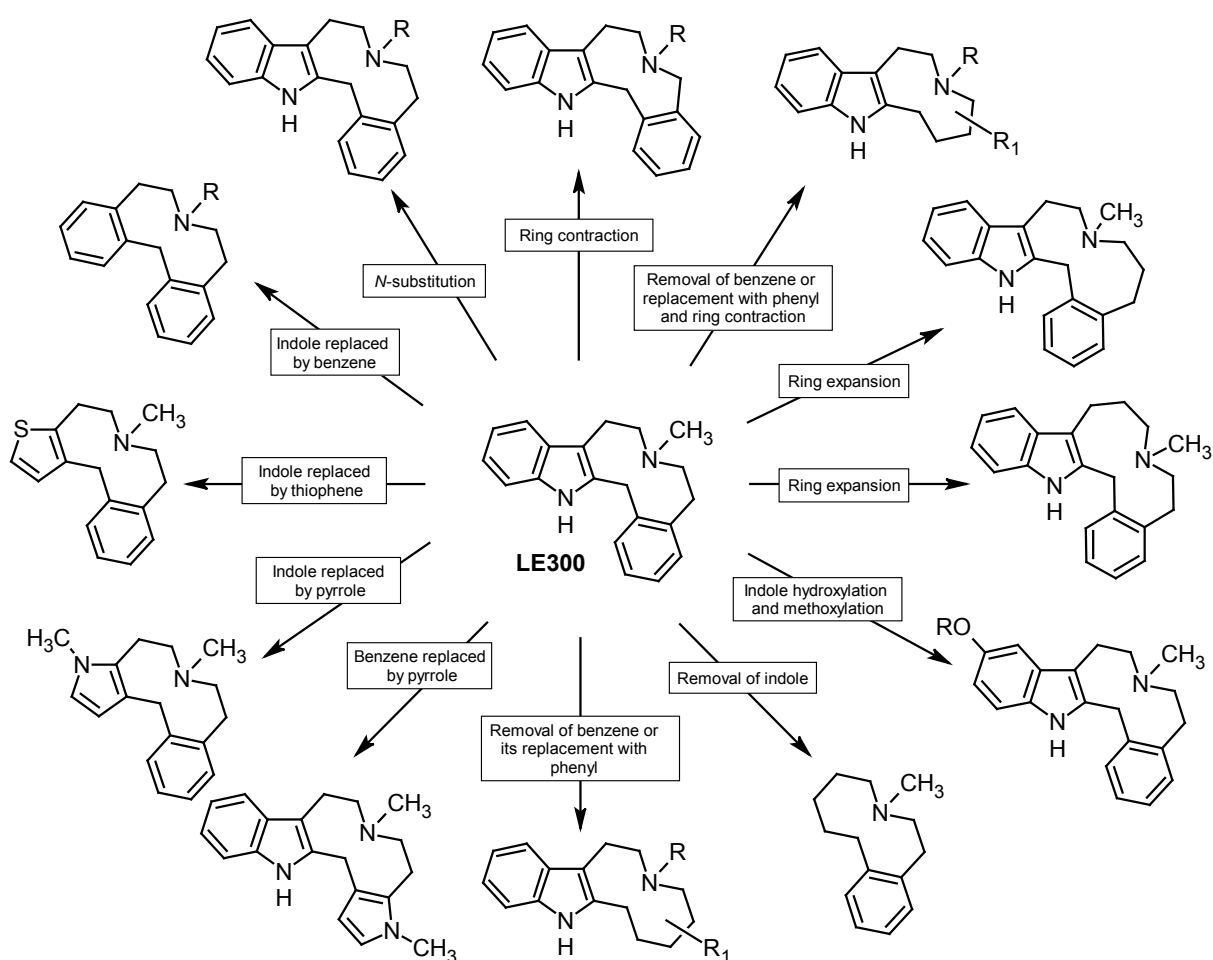
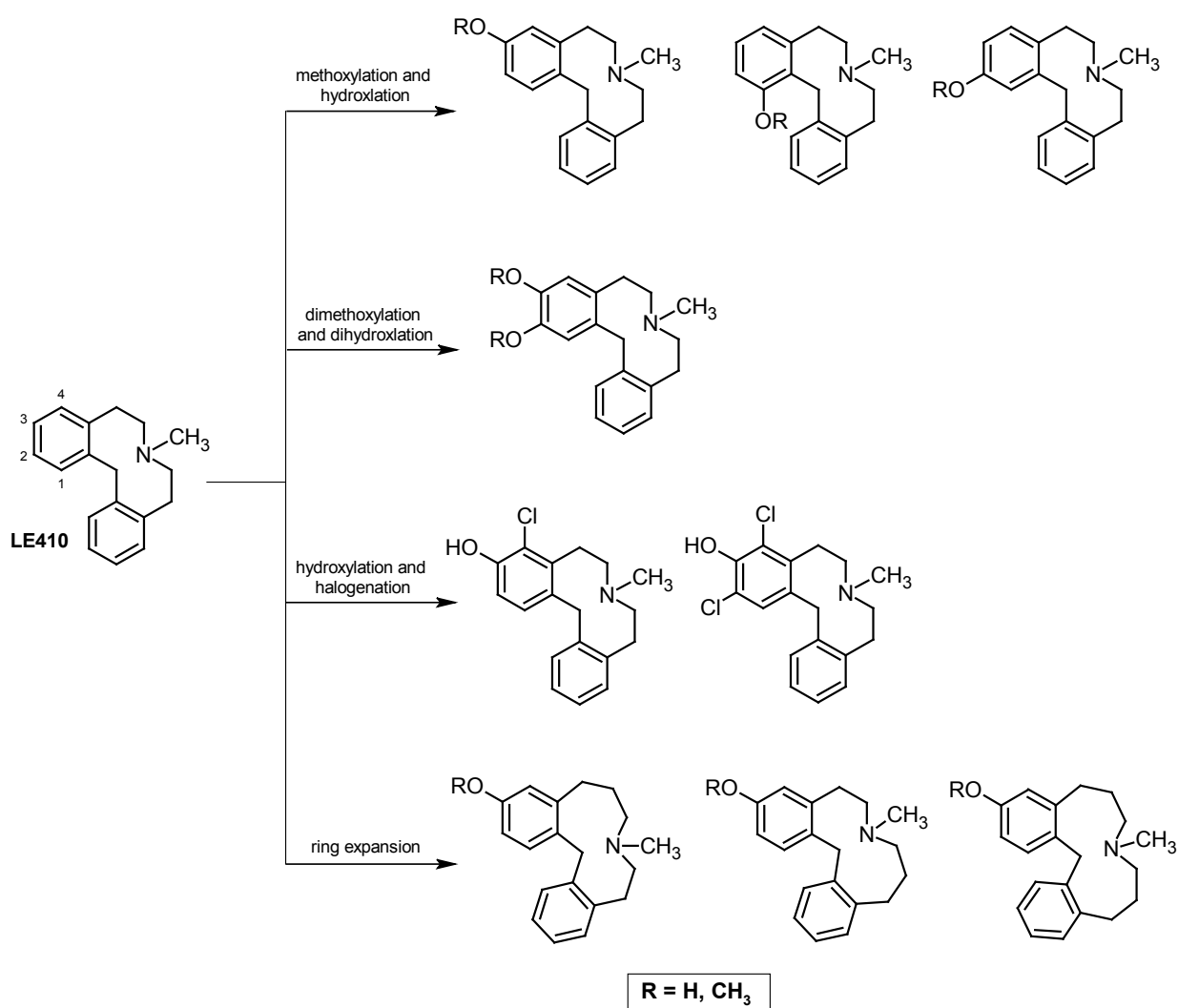


Figure 1.11: Structural modifications of LE300.

Numerous modifications have also been carried out on the structure of the dibenzazecine LE410. (**Figure 1.12**)



**Figure 1.12:** Different substitution and other structural modifications of the dibenzazecine derivatives.

These led to the following conclusions:

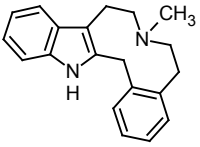
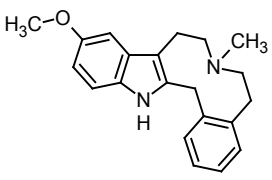
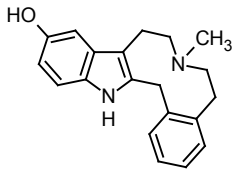
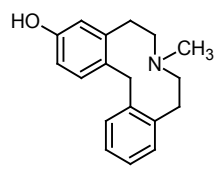
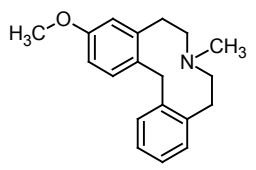
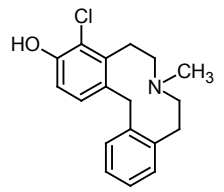
- Hydroxylated dibenzazecines mostly showed higher affinities than their methoxylated counterparts. With respect to the position of the hydroxyl and methoxy groups, the 3-hydroxy and 3-methoxy derivatives were the most active ones<sup>109</sup>. Phenolic dibenzazecines were generally more active than the methoxylated counterparts.
- 2,3-Dihydroxy- and dimethoxydibenzazecines showed much lower affinities than the monohydroxylated ones. This is quite surprising since the dihydroxylated derivative shares the same substitution pattern as dopamine. Moreover both compounds were found to display antagonistic properties at the dopamine receptors<sup>106</sup>.



- The most active compound within this series was the 4-chloro-3-hydroxydibenzazecine, which exhibited very high affinities towards the D<sub>1</sub>-like receptors and picomolar ones for the D<sub>5</sub> subtype; exhibiting a 10-15 fold selectivity for D<sub>5</sub> over D<sub>1</sub><sup>109</sup>.
- Expansion of the central azecine ring to an 11-membered ring was more or less tolerated, again depending on the site of ring elongation. A hydroxyphenethyl moiety combined with a phenylpropyl one was more favored than the combination of hydroxyphenylpropyl and phenethyl moieties<sup>110</sup>.

**Table 1.1:** K<sub>i</sub> values of various dopamine receptor ligands for the D<sub>1</sub>-D<sub>5</sub> receptors.

Code	Structure	K <sub>i</sub> (nM), (Radioligand binding experiments)				
		D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
Clozapine*		189	431	646	39	235
SCH23390*		0.35	2145	>10000	3560	0.3
SCH39166 <sup>87</sup> (ecopipam)		1.2	980	n.d.	80	2
NNC756 <sup>85</sup>		0.17	942	n.d.	n.d.	n.d.
NNC112 <sup>85</sup>		0.18	898	n.d.	n.d.	n.d.
NNC687 <sup>85</sup>		5.8	>10000	n.d.	n.d.	n.d.
Stepholidine*		5.9	974	30	3748	4

<b>LE300</b> <sup>106</sup>		1.9	44.5	40.3	109	7.5
<b>LE-CE550</b> <sup>107</sup>		0.82	11.9	475	266	3.6
<b>LE-CE551</b> <sup>107</sup>		0.56	38.4	944	398	0.39
<b>LE404</b> <sup>106</sup>		0.39	17.5	47.5	11.3	1.5
<b>LE425</b> <sup>106</sup>		28.5	13	75.7	43.3	54
<b>LE-PM436</b> <sup>109</sup>		0.83	4.0	24.6	4.7	0.06

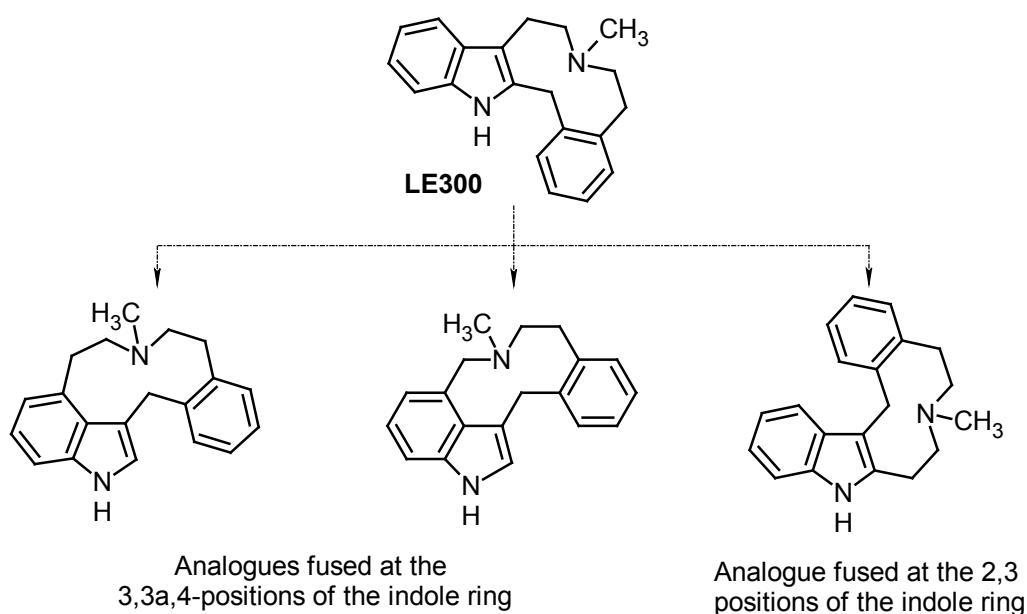
n.d. = not determined; \*Values taken from PDSP<sup>17</sup> database.

## 2. Aim of this work

Partially hydrogenated indolo[3,2-*f*][3]benzazecines and dibenz[*d,g*]azecines are a novel class of dopamine receptor antagonists, distinguished by their nanomolar affinities as well as their preference for the receptors of the D<sub>1</sub>-family. The objective of the current work was to introduce further modifications on the structures of the lead compounds LE300 (an indolo[3,2-*f*][3]benzazecine) and LE404 (a dibenz[*d,g*]azecine) in order to assess the impact of these structural modifications on the affinities for the different dopamine receptor subtypes. This will help further analyze the SAR of this distinctive class of dopaminergic ligands. A detailed description of the work project will be discussed subsequently.

### 2.1. Alteration of the annulation pattern of LE300

Goal of this part was to prepare LE300 analogues with a modified structural skeleton in order to examine the effect of the scaffold structure on the affinities and the selectivities for the dopamine receptors. The main structural features of the lead LE300 were to be maintained, namely a central aza-alicyclic ring with a benzene and an indole ring on both sides, while the fusion of the central aza-alicyclic ring with respect to the indole moiety was to be varied. The first target compounds were LE300 analogues with the central ring fused not to the 3,2- but to the 3,3a,4-positions of the indole ring.



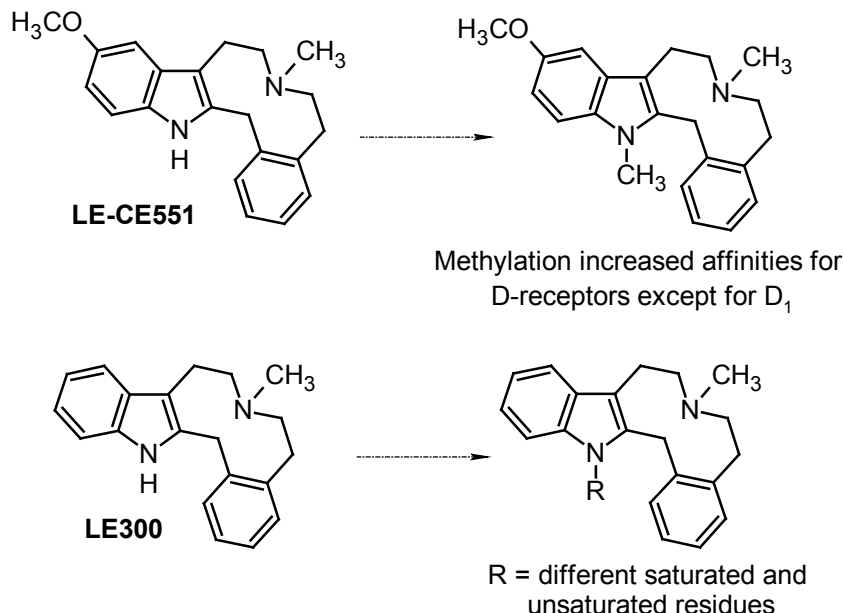
**Figure 2.1:** LE300 analogues with modified annulation patterns.

Here two different compounds came into question: First, the more rigid analogue, which encompasses a central 10-membered ring like LE300, but where the alicyclic nitrogen is connected to the indole ring by a methylene rather than an ethylene group. Second, the more flexible analogue, comprising an expanded central ring (aza-cycloundecan ring), but where an ethylene chain connects the alicyclic nitrogen to the indole ring, like in LE300. (**Figure 2.1**)

A further target compound was an indolobenzazecine derivative, where the central azecine ring is fused at the 2,3- position of the indole ring, as opposed to the 3,2-fusion pattern of LE300. (**Figure 2.1**)

## 2.2. Indole-NH substitution

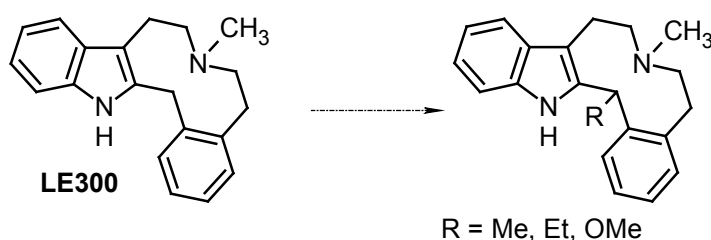
In previous investigations, methylation of the indole-NH of LE-CE551 was found to result in a significant increase in the affinities for all dopamine receptor subtypes, except for  $D_1$ <sup>107</sup>. Goal of this part was to prepare LE300 derivatives substituted at the indole-NH with a methyl group besides a series of other residues in order to test the effect of chain length, bulk and electronic properties on the activity and selectivity of the compounds. (**Figure 2.2**)



**Figure 2.2:** Indole-N substitution of LE300.

### 2.3. Placing substituents between the aromatic moieties

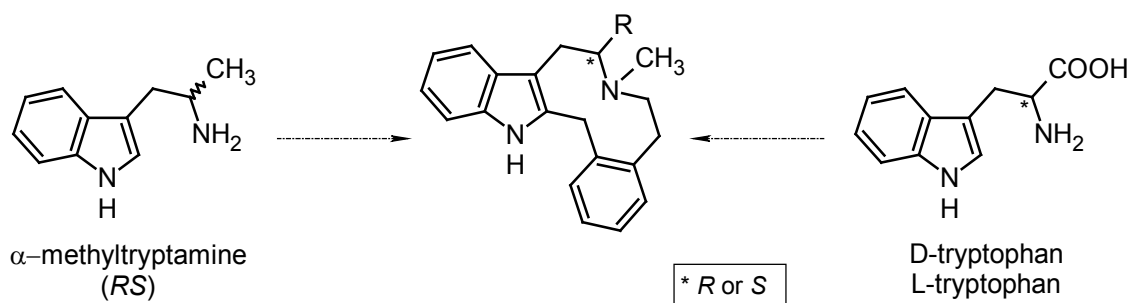
Substituents at the indole-NH might have an impact on the conformation of the different azecines, which can in turn affect the activities. In order to further explore the effect of the substituents on the conformation of the derivatives and sequentially on the affinities, it was planned to prepare derivatives bearing small substituents at the methylene bridge, connecting both aromatic moieties. These substituents should include methyl, ethyl and methoxy groups. (**Figure 2.3**)



**Figure 2.3:** Substitution of LE300 at the methylene group connecting both aromatic moieties.

### 2.4. LE300 substituted at the 8-position

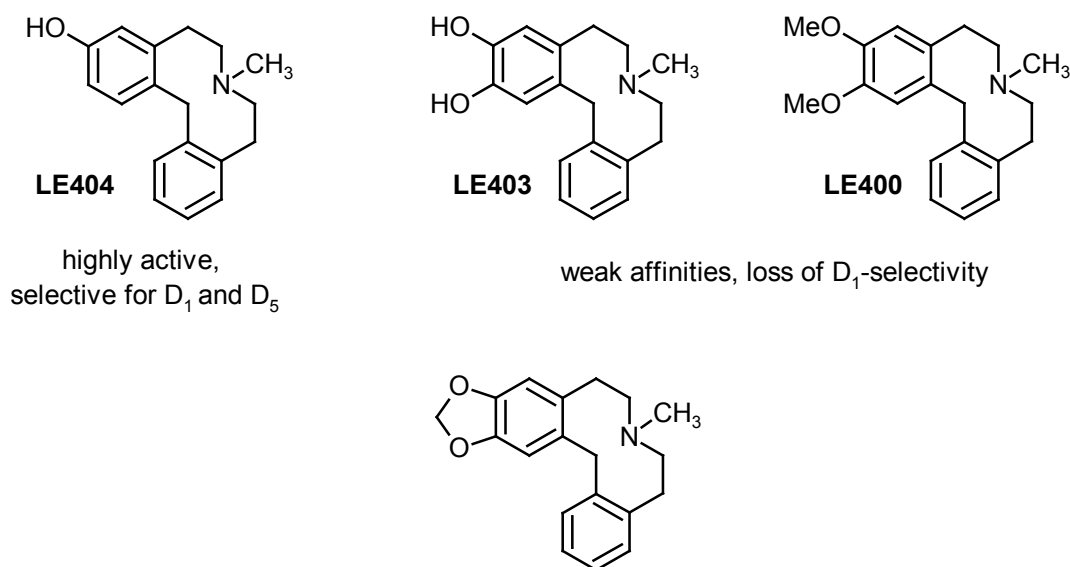
Starting from both  $\alpha$ -methyltryptamine as well as L- and D-tryptophan we aimed to prepare the corresponding indolobenzazecine derivatives and to subsequently derivatize them. (**Figure 2.4**) Here, it was attractive to obtain the chiral target compounds in an enantiopure form in order to be able to assess the effect of the stereochemistry in addition to the nature of the substituent placed at this position. The influence of the stereochemistry of the derivatives on the affinities could help better explore the spatial requirements of the binding sites of the different receptor subtypes.



**Figure 2.4:** Indolobenzazecine derivatives starting from  $\alpha$ -methyltryptamine, D- and L-tryptophan.

### 2.5. Dibenzazecine substituted with a methylenedioxy moiety

LE404 is a potent dopamine receptor antagonist, displaying high affinities and a pronounced selectivity for the  $D_1$ -like receptors. On the other hand, the 2,3-dihydroxylated and -dimethoxylated counterparts (**figure 2.5**) showed surprisingly low affinities. In case of the dimethoxy derivative this could be attributed to the fact that two methoxy substituents are spatially too bulky for the receptor binding site. Hence, we aimed to prepare an analogous dibenzazecine derivative, which is substituted at the 2 and 3 positions by a methylenedioxy group (**figure 2.5**), which requires less space due to its restricted rotation. This target derivative is postulated to show better pharmacokinetics than the phenolic dibenzazecine derivative LE404, which has a low oral bioavailability, most probably due to its fast biotransformation.



**Figure 2.5:** Changing the substitution pattern of LE404, LE403 and LE403 into a methylenedioxy derivative.

### 2.6. Antipsychotic potential of benzindoloazecines

Since many of the previously prepared azecines have shown high *in vitro* affinities not only for the dopamine, but also for serotonin  $5HT_{2A}$  receptors<sup>111</sup>, it was interesting to test their *in vivo* potential as antipsychotics. For this purpose some azecine derivatives, exhibiting high affinities and low cytotoxicity, should be selected together with the lead compounds LE300 and LE404 to carry out *in vivo* study (rat model) for antipsychotic activity as a collaborative work within the research group.

### **3. Manuscripts**





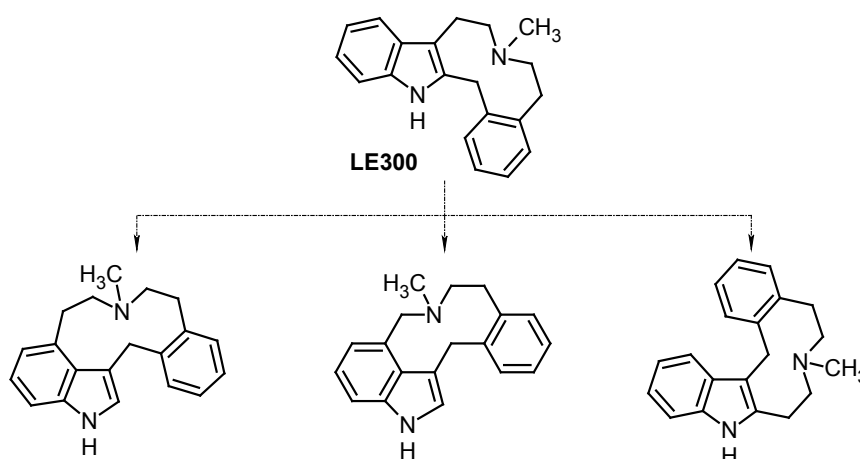
## Paper 1

### Dopamine Receptor Ligands. Part 18: Modification of the Structural Skeleton of Indolobenzazecine-Type Dopamine Receptor Antagonists

Dina Robaa, Christoph Enzensperger, Shams El Din Abul Azm, El Sayeda El Khawass, Ola El Sayed, and Jochen Lehmann

*J. Med. Chem.* **2010**, 53, 2646–2650

**Abstract:** The synthesis and affinities of three novel LE300 analogues with altered annulation patterns of the heterocyclic scaffold are reported. Results revealed that the backbone scaffold of these novel derivatives did not have a marked effect of the activity. Two derivatives, namely those where the indole ring is connected through an ethylene moiety to the central nitrogen, showed similar or slightly decreased affinities than the lead LE300, though with a rather changed selectivity profile. Meanwhile, the more rigid analogue, where the indole is connected through a methylene rather than an ethylene moiety to the alicyclic nitrogen was devoid of activity. This reveals that the degree of structural flexibility together with the distances between the pharmacophoric groups are decisive structural elements and have a major effect on the affinities of this class of dopamine antagonists.



**Contribution:** Synthesis and characterization of all target and intermediary compounds, development of SAR and preparation of the manuscript.



## Dopamine Receptor Ligands. Part 18:<sup>1</sup> Modification of the Structural Skeleton of Indolobenzazecine-Type Dopamine Receptor Antagonists

Dina Robaa,<sup>†</sup> Christoph Enzensperger,<sup>†</sup> Shams El Din Abul Azm,<sup>‡</sup> El Sayeda El Khawass,<sup>‡</sup> Ola El Sayed,<sup>‡</sup> and Jochen Lehmann<sup>\*,†</sup>

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Received August 28, 2009

On the basis of the D<sub>1/5</sub>-selective dopamine antagonist LE 300 (**1**), an indolo[3,2-*f*]benzazecine derivative, we changed the annulation pattern of the heterocycles. The target compounds represent novel heterocyclic ring systems. The most constrained indolo[4,3a,3-*ef*]benzazecine **2** was inactive, but the indolo[4,3a,3-*fg*]benzazacycloundecene **3** showed antagonistic properties (functional Ca<sup>2+</sup> assay) with nanomolar affinities (radioligand binding) for all dopamine receptor subtypes, whereas the indolo[2,3-*f*]benzazecine **4** displayed a selectivity profile similar to **3** but with decreased affinities.

### Introduction

Dopamine is a major neurotransmitter of the brain involved in the control of movement, emotion, reward, and cognition. Dysfunctions of the dopaminergic system have been associated with several neuropsychiatric disorders including Parkinson's disease, schizophrenia, and drug dependence. The azecine-type dopamine receptor antagonist 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (**1**, LE 300)<sup>2–4</sup> represents a novel chemical structure, possessing high affinities and a unique selectivity profile for the D<sub>1</sub>-like receptors. Extensive SAR studies have been carried out based on **1** (LE 300) as a lead. The objective of the present study was to rearrange the heterocyclic skeleton of our lead compound **1** in order to assess how different annulation patterns would affect the affinities and selectivities for the dopamine receptors. While retaining the basic structural features of our lead, namely an indole, a benzene ring, and a central *N*-methylated azacycloalkane ring, we mainly wanted to change the annulation of the central alicycle to the indole ring (Chart 1).

Our first targets were analogues of **1**, where the aza-alicyclic is fused not to the 2,3- but to the 3,3a,4-positions of the indole. As this annulation pattern might impart some rigidity on the structure, we wanted to synthesize both the indolo-benzazecine **2** and the more flexible indolo-benzazaundecene **3**. Compound **2** resembles the parent compound **1** in containing a 10-membered central alicycle but differs from it in containing a methylene rather than an ethylene chain, connecting the indole ring to the central nitrogen. Conversely, in the ring-expanded structure of compound **3**, the indole ring is connected to the central nitrogen through an ethylene chain. These target compounds, together with their pentacyclic precursors **8** and **12**, are of additional interest due to their structural resemblance to ergolines, especially to the selective

D<sub>1</sub> (partial)agonist (–)-4,6,6a,7,8,12b-hexahydro-7-methyl-indolo[4,3-*ab*]phenanthridine CY 208–243<sup>5,6</sup> (Chart 2). This benzergrone derivative showed 10-fold selectivity for D<sub>5</sub> over D<sub>1</sub> receptor<sup>7</sup> and exhibited in vivo antiparkinsonian activity.<sup>8</sup>

Our second target compound was the indolo-benzazecine **4**, where the 10-membered central alicycle is fused at the 2,3-positions of the indole ring as opposed to the 3,2-fusion pattern of **1** (Chart 1).

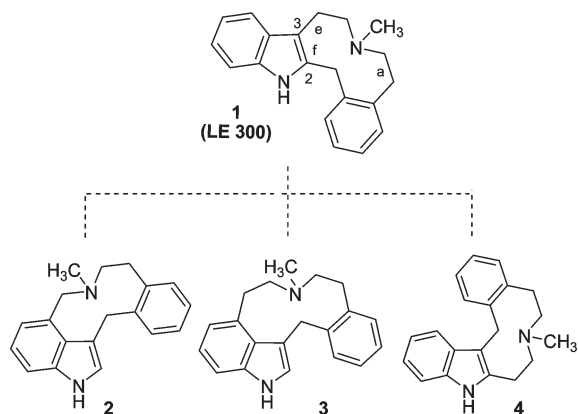
### Chemistry

To obtain the annulated 10- and 11-membered heterocycles, we had to prepare their ring-closed quinolizine-type precursors. Previously applied methods for the preparation of related quinolizines, namely by reacting the respective aralkylamines with 1-isochromanone<sup>4,9</sup> or with 2(2-chloroethyl)-benzoyl chloride,<sup>10,11</sup> were unsuccessful. So the quinolizine **8** was prepared following a procedure described by Browne for the preparation of analogous benzo-thieno-quinolizine.<sup>12</sup> Reacting 4-aminomethylindole (**5**) with 2-(2-bromoethyl)benzaldehyde (**6**) in dioxane at room temperature yielded the 3,4-dihydroisoquinolinium salt **7**. This was refluxed in 6*N* HCl, and the obtained quinolizinium salt was alkalized to give the quinolizine **8**. Two different procedures yielded the indolo-benzazecine **2**. First, the quinolizine **8** was quaternized with methyl iodide and the central C–N bond of the resulting quaternary salt **9** was subsequently cleaved by treatment with sodium in liquid ammonia. Second, the quinolizine **8** was converted with ethylchloroformate/NaCNBH<sub>3</sub> to the carbamate derivative **10**, which upon reduction with lithium aluminum hydride also yielded the desired target compound **2**. Spectral data of the products, obtained by both methods, were identical. However, the first route proved to be more advantageous with respect to the yield (Scheme 1).

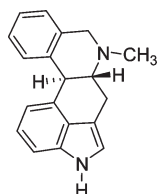
Applying the procedure, previously described for the synthesis of the quinolizine **2**, failed to produce both the homoquinolizine **12** and quinolizine **15**. 2-(2-Bromoethyl)benzaldehyde (**6**) did not react with the respective amines **11** and **14**

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**Chart 1.** Rearranging the Indolo[3,2-*f*]benzazecine Skeleton of **1** to Analogue Indolo[4,3a,3-*ef*]benzazecine **2**, Indolo[4,3a,3-*fg*]benzazaundecene **3** and to Indolo[2,3-*f*]benzazecine **4**



**Chart 2.** Selective D<sub>1</sub> (Partial) Agonist (–)-4,6,6a,7,8,12b-Hexahydro-7-methylindolo[4,3-*ab*]phenanthridine



at room temperature. Hence, we modified the reaction conditions. 4-(2-Aminoethyl)indole (**11**) or 2-(2-aminoethyl)indole (**14**) were refluxed with 2-(2-bromoethyl)benzaldehyde (**6**) in dioxane in the presence of equimolar amounts of trifluoroacetic acid. This resulted in the direct formation of the salts of the homoquinolizine **12** and quinolizine **15**, respectively. Alkalinization yielded the corresponding bases, which were quaternized with methyl iodide. Finally the obtained quaternary salts **13** and **16** were cleaved using sodium in liquid ammonia to produce the target compounds **3** and **4** (Scheme 2).

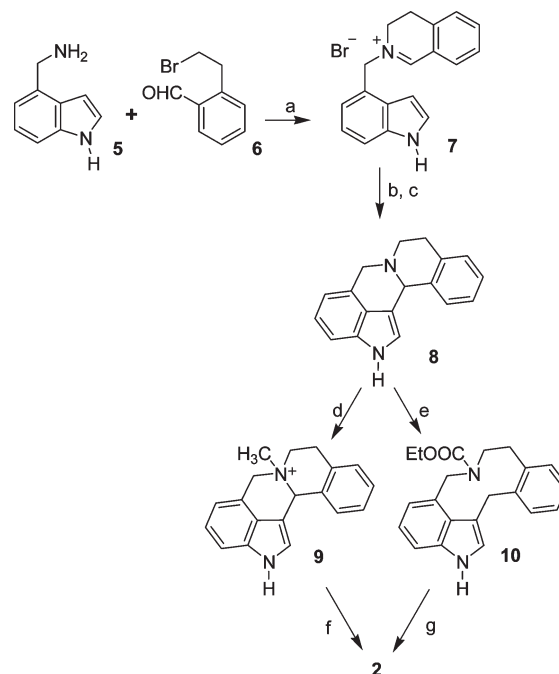
### Pharmacology

All the target compounds **2**, **3**, and **4** together with their pentacyclic precursors **8**, **12**, and **15** were screened for their affinities for the human cloned dopamine receptor subtypes D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, and D<sub>5</sub>. These receptors were stably expressed in HEK293 or CHO cells; [<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone were used as radioligands at the D<sub>1</sub>-like and D<sub>2</sub>-like receptors, respectively. *K<sub>i</sub>* values are given in nanomolar units (Table 1). For a detailed protocol, see Supporting Information (SI). Additionally, the compounds were tested in an intracellular Ca<sup>2+</sup> assay, in order to determine their functionality at the D<sub>1</sub> and D<sub>2</sub> receptors. HEK293 cells stably expressing the respective D-receptor were loaded with a fluorescent dye, and after preincubation with rising concentrations of the test compound, an agonist (SKF 38393 for D<sub>1</sub> and quinpirole for D<sub>2</sub>) was injected and the Ca<sup>2+</sup>-induced fluorescence was measured with a microplate reader. The ability of the test compound to suppress the agonist-induced Ca<sup>2+</sup> influx is an indication of antagonistic or inverse agonistic properties at the receptor. For a detailed description, see SI.

### Results and Discussion

We synthesized three analogues of the lead compound **1**, possessing a modified annulation pattern. All of these

**Scheme 1.** Total Synthesis of 5-Methyl-4,5,6,7-tetrahydroindolo[4,3a,3-*ef*][3]benzazecine (**2**)<sup>a</sup>

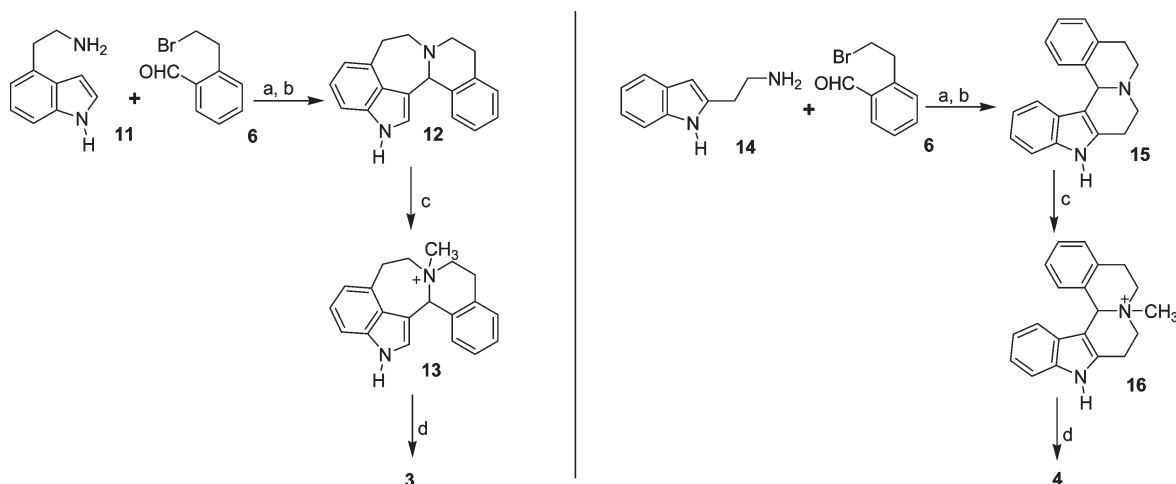


<sup>a</sup> Reagents and conditions: (a) dioxane, rt, 3 h; (b) 6 N HCl, reflux, 4 h; (c) aq NH<sub>4</sub>OH; (d) MeI, acetone, rt, 24 h; (e) ClCOOEt, dry THF, –55 °C, 5 h, then NaCNBH<sub>3</sub>, –55 °C to rt; (f) Na<sup>0</sup>, liquid NH<sub>3</sub>, –40 °C, 10 min; (g) LiAlH<sub>4</sub>, dry THF, reflux, 3 h.

compounds, together with their pentacyclic precursors, are derivatives of novel heterocyclic ring systems.

In the radioligand binding experiments, the two homologues, **2** and **3**, where the alicycle is fused at the 3,3a,4 positions of the indole ring, were found to possess surprisingly different affinities: while the indolo[4,3a,3-*ef*]benzazecine **2** showed no significant affinities for all dopamine receptor subtypes (*K<sub>i</sub>* > 10 μM), the ring expanded indolo[4,3a,3-*fg*]benzazaundecene **3** displayed nanomolar affinities, comparable to those of our lead **1**. However, it showed almost equal affinities for the D<sub>1</sub>-, D<sub>3</sub>-, and D<sub>5</sub>-receptor, showing a slightly higher affinity for D<sub>5</sub> compared to D<sub>1</sub>. A 10-fold increase in the affinity for D<sub>2</sub> was observed, when compared to our lead **1**, in addition to an improvement in the affinity for the D<sub>4</sub>-receptor. The indolo[2,3-*f*]benzazecine **4** showed a nearly similar selectivity pattern like that of compound **3** but with noticeable decrease in the affinities for all D-receptors. Although both compound **2** and our lead **1** contain a benzindolo-azecine moiety, the structure of the former is much more constrained as a consequence of its annulation. In contrast, the ring expanded structure of compound **3** shows higher flexibility, which is accompanied by an extreme increase in affinities. In accordance with our previous studies,<sup>4</sup> only structures showing a certain degree of flexibility display relevant affinities for the dopamine receptors.

The discrepancy in the activities between both homologues **2** and **3** might be attributed to the different distances between the pharmacophoric groups, which are the central N and both aromatic rings, but also to the different angles between the aromatic rings in both structures. Another possibility may be that analogue **2** is inactive due to the nitrogen's lone e-pair being buried in the center of the ring beneath the *N*-methyl group. Meanwhile, the flexibility of the additional methylene

**Scheme 2.** Total Synthesis of Benzazacycloundecene **3** and Benzazecine **4**<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) trifluoroacetic acid, dioxane, reflux, 20 h; (b) 2 N NaOH; (c) MeI, acetone or acetonitrile, rt, 24 h; (d) Na<sup>+</sup>, liquid NH<sub>3</sub>, -40 °C, 10 min.

**Table 1.** Affinities ( $K_i$ , nM) for Human D<sub>1</sub>–D<sub>5</sub> Receptors, Determined by Radioligand Binding Experiments

compd	$K_i$ (nM)				
	HEK D <sub>1</sub>	HEK D <sub>2L</sub>	HEK D <sub>3</sub>	CHO D <sub>4,4</sub>	HEK D <sub>5</sub>
<b>1</b> <sup>a</sup> (LE 300)	1.9 ± 0.9	44.5 ± 15.8	25.9	108 ± 39	7.5 ± 0.3
<b>2</b>	> 10000	> 10000	> 10000	> 10000	> 10000
<b>3</b>	4.2 ± 0.5 <sup>b</sup>	4.0 ± 0.5 <sup>c</sup>	42.9 ± 8.5 <sup>c</sup>	39.1 ± 9.6 <sup>b</sup>	2.5 ± 0.7 <sup>c</sup>
<b>4</b>	28.5 ± 7.2 <sup>b</sup>	55.1 ± 15.4 <sup>c</sup>	513 ± 217 <sup>b</sup>	225 ± 50 <sup>c</sup>	10.3 ± 1.5 <sup>c</sup>
<b>8</b>	> 10000	> 10000	> 10000	> 10000	> 10000
<b>12</b>	1836 ± 658 <sup>b</sup>	4458 ± 1192 <sup>c</sup>	> 10000	> 10000	1525 ± 206 <sup>c</sup>
<b>15</b>	2101 ± 703 <sup>b</sup>	1476 ± 28.5 <sup>c</sup>	> 10000	> 10000	> 10000

<sup>a</sup> Values cited from ref 10. CHO cell lines were used for D<sub>2L</sub>, D<sub>3</sub>, and D<sub>4,4</sub>; HEK cell lines for D<sub>1</sub> and D<sub>5</sub>. <sup>b</sup>  $K_i$  values are the means of three experiments; performed in triplicate ± SEM. <sup>c</sup>  $K_i$  values are the means of two; experiments; performed in triplicate ± SEM.

in analogue **3** might permit low-energy conformations to exist with the N lone e-pair to be directed outward. However, studies have shown that the receptor binding site contains two aromatic subsites around a conserved Asp<sup>3,32</sup>, whose carboxylate group acts as a counterion of the protonated basic N of the ligand.<sup>13</sup> Accordingly, we expect our ligands to interact with the binding sites in an N-protonated form and not with the N lone pair of the free base.

The pentacyclic precursor compounds **8**, **12**, and **15** had generally weak to no affinities. The quinolizine **8** displayed no affinities ( $K_i > 10 \mu\text{M}$ ) for all dopamine receptors, whereas its homologue **12** showed micromolar ones for the D<sub>1</sub>, D<sub>2</sub>, and D<sub>5</sub> subtypes. Similarly, the quinolizine **15** showed micromolar affinities for the D<sub>1</sub> and D<sub>2</sub> subtypes. With the exception of the quinolizine **2**, removal of the C–N bond of the quinolizines and the consequent impairment of a moderate flexibility to the central ring results in a dramatic increase in affinities. This is in accordance with some previous observations.<sup>4</sup>

All tested compounds, displaying affinities in the radioligand binding assay, were found to possess antagonistic activities at the D<sub>1</sub> and D<sub>2</sub> receptors in the calcium assay.

We conclude that modifying the annulation pattern of the parent compound **1** does not result in significant changes in the affinities for the different dopamine receptors; equally active (compound **3**) or slightly less active (compound **4**) dopamine antagonists have been obtained. It seems, however, that decreasing the flexibility of the structure together with decreasing the distances between the alicyclic nitrogen and the aromatic moieties both have a negative effect on the affinities and can lead to a complete loss of activity.

## Experimental Section

**General Methods.** Melting points are uncorrected and were measured in open capillary tubes using a Gallenkamp melting point apparatus. <sup>1</sup>H and <sup>13</sup>C NMR spectral data were obtained from a Bruker Avance 250 spectrometer (250 MHz) and Avance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS using a Hewlett-Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Purities of the compounds were determined by elemental analysis, performed on a Hereaus Vario EL apparatus. All values for C, H, and N were found to be within ±0.4. All compounds showed >95% purity.



**2,8,9,13b-Tetrahydro-6H-isoquinolo[2,1-b]pyrrolo[4,3-de]-isoquinoline (8).** To a stirred solution of 4-aminomethylindole<sup>14</sup> (1.16 g, 8 mmol) in 30 mL dioxane was added dropwise a solution of 2-(2-bromoethyl)benzaldehyde<sup>15</sup> (3.4 g, 16 mmol) in 10 mL dioxane. Stirring was continued for 3 h, whereupon the intermediate isoquinolinium salt separated as a brownish sticky substance. The mixture was then allowed to settle, the supernatant was decanted, the sediment washed twice with dioxane and then twice with diethyl ether, and finally dried under vacuum. The obtained isoquinolinium salt **7** was subsequently, without further purification, dissolved in 30 mL 6 N HCl and the solution was refluxed for 4 h. The formed quinolinizinium salt, which separated as white solid, was filtered off, washed several times with cold water, and dried. It was then alkalized with aqueous ammonia and the formed quinolizine base **8** was extracted with a mixture of dichloromethane/isopropanol (3:1). Finally, the extract was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvents removed under reduced pressure to give 0.86 g (41.1%) of a white solid. This was recrystallized from chloroform/ethanol giving cream-white crystals; mp: 185–188 °C. <sup>1</sup>H NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 2.64–3.01 (m, 4H, 8, 9), 3.93–3.99 (d, *J* = 15.85, 1H, 6), 4.31–4.37 (d, *J* = 15.85, 1H, 6), 5.33 (s, 1H, 13b), 6.73–6.76 (m, 2H, Ar-H), 6.98–7.04 (t, 1H, *J* = 7.4, Ar-H) 7.16–7.27 (m, 4H, Ar-H), 7.41–7.44 (d, *J* = 7.1, 1H, 5), 10.66 (s, 1H, indole-NH). <sup>13</sup>C NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 29.43 (9), 46.48 (8), 55.34 (6), 57.8 (13b), 109.48 (3), 113.53 (13c), 113.92 (1), 120.19 (5), 122.51 (11), 124.83 (5b), 126.03 (12), 126.54 (4), 127.24 (13), 128.13 (5a), 129.35 (10), 133.93 (9a), 134.03 (2a), 137.77 (13a). Anal. (C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>·0.25EtOH): C, H, N.

**General Procedure for the Preparation of Homoquinolizine 12 and Quinolizine 15.** A solution of 4-(2-aminoethyl)-<sup>16</sup> or 2-(2-aminoethyl)indole<sup>17</sup> (7.5 mmol), 2-(2-bromoethyl) benzaldehyde (9.4 mmol), and trifluoroacetic acid (7.5 mmol) in dioxane was refluxed under nitrogen for 20 h, whereupon a solid formed. The precipitated salts were filtered off, washed with dioxane and then with diethyl ether, and subsequently dissolved in hot water, filtered, and the filtrate rendered alkaline with 1 N NaOH. Finally, the formed bases were extracted with chloroform and the extracts dried over Na<sub>2</sub>SO<sub>4</sub>.

**2,6,7,9,10,14b-Hexahydroindolo[3',4':3,4,5]azepino[2,1-a]-isoquinoline (12).** Evaporation of the solvent yielded a brown solid that was crystallized from isopropanol/ether and then recrystallized from chloroform to give cream colored crystals; crude yield 89%; mp: 188–190 °C. <sup>1</sup>H NMR for the base: 250 MHz (CDCl<sub>3</sub>): δ 2.75–3.63 (m, 8H, 6, 7, 9, 10), 5.42 (s, 1H, 14b), 6.55 (s, 1H, 1), 6.91–6.94 (d, *J* = 6.9, 1H, 11), 7.09–7.27 (m, 6H, 3, 4, 5, 12, 13, 14), 8.04 (s, 1H, indole-NH). <sup>13</sup>C NMR for HCl salt: 400 MHz (MeOD-*d*<sub>4</sub>): δ 25.60 (10), 28.09 (6), 57.82 (7), 59.39 (9), 66.74 (14b), 109.54 (14c), 110.02 (3), 118.57 (1), 122.64 (4), 125.36 (5b), 126.36 (5), 126.67 (12), 128.16 (14), 128.56 (13), 128.89 (11), 130.58 (5a), 131.19 (14a), 131.94 (10a), 136. (2a). Anal. (C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>·0.5CHCl<sub>3</sub>): C, H, N.

**5,6,8,9,10,14c-Hexahydroindolo[3',2':3,4]pyrido[2,1-a]isoquinoline (15).** Evaporation of the solvent yielded an orange-brown solid, which was purified by column chromatography (EtOAc–MeOH, 15:1); yield 21%; mp: 197–200 °C (analytical data see SI).

**General Procedure for the Preparation of the Quaternary Salts.** To a stirred solution of the respective quinolizine or homoquinolizine in acetone (or acetonitrile for compound **15**) was added a 10-fold molar excess of methyl iodide. Stirring was continued for a period ranging from 24 to 48 h, and the formed quaternary salts were isolated (analytical data, see SI).

**General Procedure for the Ring-Opening.** Ammonia was condensed in a three-necked 100 mL flask, which was equipped with a balloon and a stopper and cooled in a liquid nitrogen bath. After filling <sup>3</sup>/<sub>4</sub> of the flask's volume, the cooling bath was removed and ammonia was allowed to liquefy. The respective quaternary salts were then added to the stirred liquid ammonia. This was followed by gradual addition of small pieces of sodium metal until the blue color remained for 10 min. A few drops of

saturated ammonium chloride solution were added to terminate the reaction, and the mixture was stirred under nitrogen until the liquid ammonia completely evaporated. To the residue was added 10 mL water, and the mixture was then extracted with 30 mL diethyl ether. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was removed under reduced pressure to yield the crude products, which (except for compound **2**) were sufficiently pure and did not require further purification.

**5-Methyl-4,5,6,7-tetrahydroindolo[4,3a,3-ef][3]benzazecine (2).** Evaporation of the solvent yielded a cream-colored solid, which was crystallized from chloroform; yield 24%; mp: 257–260 °C. <sup>1</sup>H NMR: 250 MHz (CDCl<sub>3</sub>): δ 1.55 (mc, 2H, 6), 2.12 (s, 3H, N-Me), 2.78 (mc, 2H, 7), 3.15 (mc, 2H, 4), 4.58 (s, br, 2H, 12), 6.73–6.76 (d, *J* = 7.1, 1H, Ar-H), 6.97–7.26 (m, 7H, Ar-H), 8.00 (s, 1H, indole-NH). HRMS 276.1621 (calcd for C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>: 276.1626). Anal. (C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>): C, H, N.

**6-Methyl-5,6,7,8,13,15-hexahydro-4H-indolo[4,3a,3-fg][3]benzazacycloundecene (3).** Evaporation of the solvent yielded a yellowish solid; yield 62%; mp: 157–159 °C. <sup>1</sup>H NMR: 250 MHz (CDCl<sub>3</sub>): δ 2.49 (s, 3H, N-Me), 2.88 (mc, 4H, 4, 8), 3.07 (mc, 4H, 5, 7), 4.42 (s, 2H, 13), 6.79–6.82 (d, *J* = 7.1, 1H, Ar-H), 7.02–7.27 (m, 7H, Ar-H), 8.39 (s, 1H, indole-NH). <sup>13</sup>C NMR: (CDCl<sub>3</sub>): δ 28.96 (4), 29.03 (8), 44.64 (N-Me), 56.72 (7), 57.66 (5), 109.63 (1), 115.15 (13), 121.24 (3), 124.68 (2), 124.68 (10), 125.61 (3b), 126.04 (14), 126.14 (12), 129.33 (11), 130.34 (9), 133.39 (3a), 137.58 (8a), 140.11 (14a) 140.89 (12a). Anal. (C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>·0.67H<sub>2</sub>O): C, H, N.

**7-Methyl-6,7,8,9,10,15-hexahydroindolo[2,3-f][3]benzazecine (4).** Evaporation of the solvent yielded a beige solid; yield 65%; mp: 139–141 °C (analytical data see SI).

**Ethyl 4,5,6,7-Tetrahydroindolo[4,3a,3-ef][3]benzazecine-5-carboxylate (10).** A stirred solution of the quinolizine **8** (0.56 g, 2.2 mmol) in 200 mL of dry THF was cooled in methanol/dry ice at –55 °C. While keeping the reaction mixture under nitrogen, ethyl chloroformate (1.26 g, 11.6 mmol) was added and stirring was continued for 5 h. Then a solution of sodium cyanoborohydride (0.46 g; 7.3 mmol) in 10 mL dry THF was added at –55 °C and the reaction mixture was stirred overnight while allowing it to reach room temperature. It was subsequently treated with 120 mL of 2 N NaOH, the THF layer was separated, washed with brine, and finally the organic layer was evaporated under reduced pressure (analytical data, see SI).

**Reduction of the Carbamate.** A solution of compound **10** (200 mg, 0.6 mmol) in 10 mL of THF was slowly added to an ice-cooled, stirred suspension of lithium aluminum hydride (100 mg, 2.6 mmol) in 15 mL of dry THF while keeping the reaction under nitrogen. After the addition was completed, the reaction mixture was heated under reflux for 3 h. It was then cooled in an ice bath, and the excess of unreacted lithium aluminum hydride was quenched by careful addition of saturated potassium sodium tartarate solution until no H<sub>2</sub> evolved. The resulting suspension was then filtered off and the filtrate evaporated under reduced pressure to yield compound **2**, which was crystallized from chloroform yielding white crystals; yield 40%; mp: 257–260 °C. <sup>1</sup>H NMR: 250 MHz (CDCl<sub>3</sub>): δ 1.56 (mc, 2H, 7), 2.13 (s, 3H, N-Me), 2.79 (mc, 2H, 6), (mc, 2H, 4), (s, br, 2H, 12), 6.73–6.76 (d, *J* = 7, 1H, Ar-H), 7.00–7.26 (m, 7H, Ar-H), 8.00 (s, 1H, NH).

**Acknowledgment.** We thank Bärbel Schmalwasser, Petra Wiecha, and Heidi Traber for skillful technical assistance in performing the pharmacological assays. We thank the Egyptian Cultural Affairs and Mission Section for their financial support. Furthermore, we thank the DFG for the financial support of the project (EN 875/1-1).

**Supporting Information Available:** Detailed synthetic procedures as well as physical and spectral data for some target and intermediary compounds; table of elemental analysis for key compounds; a detailed protocol for the pharmacological assays.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

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# Supporting Information

## Dopamine Receptor Ligands. Part 18<sup>1</sup>: Modification of the Structural Skeleton of Indolobenzazecine-Type Dopamine Receptor Antagonists

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- I. Experimental synthetic procedures
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## I. Experimental synthetic procedures:

**General methods:** Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz). Elemental analyses were performed on a Hereaus Vario EL apparatus. TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific).

### 7-Methyl-6,7,8,9,10,15-hexahydroindolo[2,3-*f*][3]benzazecine (4):

$^1\text{H}$ -NMR: 400 MHz ( $\text{CDCl}_3$ ):  $\delta$  2.31 (s, 3H, N-Me), 2.65 - 2.67 (t,  $J = 6$ , 2H, 9), 2.73 - 2.77 (m, 4H, 6, 8), 2.88 - 2.91 (t,  $J = 6$ , 2H, 5), 4.32 (s, 2H, 15), 7.08 - 7.16 (m, 4H, Ar-H), 7.27 (dd, 1H, Ar-H), 7.43 (dd, 1H, Ar-H), 7.67 - 7.70 (dt, 2H, Ar-H).  $^{13}\text{C}$ -NMR: 400 MHz ( $\text{CDCl}_3$ ):  $\delta$  27.19 (9), 29.38 (5), 34.39 (15), 45.84 (N-Me), 57.98 (6), 58.65 (8), 110.17 (11), 112.59 (14b), 118.57 (14), 119.14 (13), 121.02 (12), 126.07 (3), 126.20 (1), 129.42 (14a), 130.27 (2), 130.78 (4), 135.02 (10a), 135.12 (4a), 140.22(9a), 140.28(15a). GC-MS:  $m/z$ : 290 (1%), 230 (3%), 218 (11%), 143 (9%), 130 (5%), 115 (7%), 104 (5%), 89 (4%), 79 (8%), 71 (9%), 57 (32%), 42 (100%). Anal. ( $\text{C}_{20}\text{H}_{22}\text{N}_2 \times 0.25 \text{H}_2\text{O}$ ): C, H, N

**4-Aminomethylindole (5):** Prepared by reduction of 4-cyanoindole using  $\text{LiAlH}_4$  according to Lit.<sup>2</sup>: A solution of 4-cyanoindole (5.68 g, 40 mmol) in 50 ml dry THF was added slowly to a stirred suspension of  $\text{LiAlH}_4$  in 200 ml dry THF, at 0 °C under nitrogen. The reaction mixture was heated under reflux for 90 minutes, and then cooled, and the excess unreacted  $\text{LiAlH}_4$  was destroyed by careful addition of a saturated potassium sodium tartarate solution. It was then filtered, and the filtrate dried over  $\text{Na}_2\text{SO}_4$ . The dried solution was finally evaporated under reduced pressure, yielding 5.55 g (yield 95.7 %) of a white solid. Mp.: 134 - 136°C, reported 124 - 125 °C;  $^1\text{H}$ -NMR: 250 MHz ( $\text{CDCl}_3$ ):  $\delta$ : 1.65 (s, 2H), 4.17 (s, 2H), 6.63 - 6.65 (mc, 1H), 7.07 - 7.10 (d, 1H), 7.19 - 7.26 (m, 2H), 7.31 - 7.34 (d, 1H), 8.44 (s, br, 1H).

**2-(2-Bromoethyl)-benzaldehyde (6):** The same procedure described in Lit.<sup>3</sup> was applied, but dichlormethane was used as a solvent instead of carbontetrachloride. The crude product was used without purification for further syntheses.

**2-(Indol-4-ylmethyl)-3,4-dihydroisoquinolinium bromide (7):** A brownish sticky substance was obtained. Samples for analytical analyses were crystallized from isopropanol/ether, giving greenish-yellow crystals. Mp.: 179-180°C. <sup>1</sup>H-NMR: 400 MHz (DMSO *d*<sub>6</sub>): 3.11 - 3.16 (t, 2H, *J* = 8, 3), 3.90 - 3.94 (t, 2H, *J* = 8, 4), 5.51 (s, 2H, N<sup>+</sup>-CH<sub>2</sub>), 6.69 (mc, 1H, Ar-H), 7.17 - 7.21 (t, 1H, *J* = 8, Ar-H), 7.25 - 7.27 (d, 1H, *J* = 7, Ar-H), 7.46 - 7.48 (t, *J* = 3, 2H, Ar-H), 7.52 - 7.54 (d, 1H, *J* = 8, Ar-H), 7.57 - 7.59 (d, 1H, *J* = 8, Ar-H), 7.79 - 7.83 (dt, 1H, *J* = 1, 8, Ar-H), 7.94 - 7.96 (d, 1H, *J* = 8, Ar-H), 9.67 (s, 1H, N<sup>+</sup>=CH), 11.43 (s, 1H, indole-NH).

**7-Methyl-2,8,9,13b-tetrahydro-6H-isoquino[2,1-*b*]pyrrolo[4,3,2-*de*]isoquinolin-7-ium iodide (9):** Creamy-white solid, Yield 91%. Samples for analysis were crystallized from a mixture of water / ethanol (4:1). Mp.: chars without melting at 263°C. <sup>1</sup>H-NMR: 400 MHz (DMSO *d*<sub>6</sub>): δ 3.26 (s, 3H, N-CH<sub>3</sub>), 3.26 – 3.37, 3.63 and 3.81 (mc, 4H, 8, 9), 5.00 – 5.04 (d, 1H, *J* = 14.9, 6), 5.13 – 5.16 (d, 1H, *J* = 14.9, 6), 6.28 (s, 1H, 13b), 7.03 – 7.04 (d, 1H, *J* = 7, Ar-H), 7.14 (s, 1H, Ar-H), 7.21 – 7.26 (t, 1H, *J* = 7.6, Ar-H), 7.38 – 7.43 (m, 5H, Ar-H), 11.25 (s, 1H, indole-NH); <sup>13</sup>C-NMR: 400 MHz (DMSO *d*<sub>6</sub>) at 343 K: 23.84 (9), 49.54 (N<sup>+</sup>-Me), 55.12 (8) (weak signal), 62.82 (6) (weak signal), 68.91 (13b), 107.87 (13c), 112.29 (3), 116.40 (2), 119.68 (5a), 120.92 (13a), 123.81 (5), 124.19 (11), 127.70 (4), 128.07 (13), 128.97 (12), 129.21 (13d), 129.72 (10), 131.00 (9a), 134.02 (2a). Anal. (C<sub>19</sub>H<sub>19</sub>IN<sub>2</sub>) C, H, N

**Ethyl 4,5,6,7-tetrahydroindolo[4,3a,3-*ef*][3]benzazecine-5-carboxylate (10):** Purified by column chromatography (hexane - EtOAc, 3:1). White solid. Yield 27 %. Mp.: 168-170 °C. <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): δ 1.06 (s, br, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 3.11 (mc, 2H, 7), 3.57 (mc, 2H, 6), 4.03 - 4.05 (d, 2H, 4), 4.63 (s, br, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 7.06 - 7.34 (m, 8H, Ar-H), 8.21 (s, 1H, indole-NH). <sup>13</sup>C-NMR 250 MHz (CDCl<sub>3</sub>): δ 14.38 (OCH<sub>2</sub>CH<sub>3</sub>), 29.61 (7), 33.04 (12), 48.5 (6), 50.85 (4), 61.11 (OCH<sub>2</sub>CH<sub>3</sub>), 110.87 (1),

115.98 (9), 121.73 (3), 122.97 (12a), 124.15 (11), 125.93 (3a), 126.3 (2), 129.88 (10), 130.25 (8), 130.58 (3b), 137.01 (7a), 137.79 (14a), 142 (11a), 156.8 (C=O). GC-MS: m/z: 334 (24%), 261 (12%), 245 (19%), 232 (50%), 218 (100%), 202 (11%), 189 (5%), 130 (21%), 115 (30%), 103 (12%), 89 (17%), 77 (28%), 63 (16%). Anal. (C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub> x 0.67 EtOAc): C, H, N

**4-(2-Aminoethyl)indole (11):** This was prepared according to the procedure described in Lit.<sup>4</sup>. Indole-4-carbonitrile was converted to indole-4-carboxaldehyde using sodium hypophosphite/Raney nickel. Condensation of the obtained carboxaldehyde derivative with nitromethane gave 4-(2-nitrovinyl)indole, which was reduced by LiAlH<sub>4</sub> to the desired amine. The crude amine, was used for subsequent syntheses. <sup>1</sup>H-NMR: 250 MHz (DMSO *d*<sub>6</sub>): 2.81 - 2.93 (m, 6H), 6.47 (s, 1H), 6.76 - 6.79 (d, 1H), 6.95 - 7.01 (t, 1H), 7.20 - 7.29 (m, 2H), 11.04 (s, 1H).

**8-Methyl-2,6,7,9,10,14b-hexahydroindolo[3',4':3,4,5]azepino[2,1-a]isoquinolin-8-ium iodide (13):** Evaporating the solvent yielded a brownish solid, which was washed with diethylether, dried and finally crystallized from water to give yellow crystals. Yield 71%. Mp.: 224-227°C. <sup>1</sup>H-NMR: 250 MHz (MeOD *d*<sub>4</sub>): δ 3.09 – 4.18 (m, 8H, 6, 7, 9, 10 and N-CH<sub>3</sub>), 6.52 (s, 2H, 1, 14b), 6.91 - 6.93 (d, *J* = 7.1, 1H, Ar-H), 7.07 – 7.43 (m, 7H, Ar-H). Anal. (C<sub>20</sub>H<sub>21</sub>IN<sub>2</sub> x 0.25 H<sub>2</sub>O): C, H, N

**2-(2-Aminoethyl)indole (14):** According to Lit.<sup>5</sup>: Ethyl indole-2-carboxylate was reduced using LiAlH<sub>4</sub> to indol-2-ylmethanol, which was oxidized by activated MnO<sub>2</sub> to indole-2-carboxaldehyde. Condensation of the latter with nitromethane afforded 4-(2-nitrovinyl)indole, which was finally reduced by LiAlH<sub>4</sub> to the desired amine. The crude amine was used for subsequent syntheses. <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): 1.77 (s, br, 2H), 2.82 - 2.87 (t, *J* = 6.1, 2H), 3.02 - 3.07 (t, *J* = 6.1, 2H), 6.27 (s, 1H), 7.09 - 7.15 (m, 2H), 7.67 - 7.32 (t, *J* = 6.9, 1H), 7.55 - 7.59 (dd, *J* = 6.7, 1H), 9.11 (s, 1H).

**7-Methyl-5,6,8,9,10,14c-hexahydroindolo[3',2':3,4,]pyrido[2,1-a]isoquinolin-7-ium iodide (16):** Evaporating the solvent yielded a yellowish-brown solid, that did not crystallize. Yield 69%. Mp.: 248-251°C. <sup>1</sup>H-NMR: 250 MHz (MeOD *d*<sub>4</sub>): δ 3.23 – 4.14 (m, 11 H, 5, 6, 8, 9 and N-CH<sub>3</sub>), 6.11 (s, 1H,

14c), 7.08 – 7.48 (m, 8H, Ar-H). <sup>13</sup>C-NMR : 250 MHz (MeOD *d*<sub>4</sub>): δ 19.04 (9), 23.70 (5), 48.58 (N<sup>+</sup>-Me), 53.51 (8), 60.45 (6), 67.22 (14c), 104.43 (14b), 11.17 (11), 117.64 (14), 119.88 (13), 122.07 (12), 126.52 (14d), 127.18 (2), 128.01 (14a), 128.06 (3), 128.32 (1), 128.67 (4), 130.02 (9a), 132.13 (4a), 137.04 (10a). Anal. (C<sub>20</sub>H<sub>21</sub>IN<sub>2</sub> x H<sub>2</sub>O): C, H, N

**5,6,8,9,10,14c-Hexahydroindolo[3',2':3,4,]pyrido[2,1-*a*]isoquinoline (15):**

<sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): δ 2.61 - 3.63 (m, 8H, 5, 6, 8, 9), 5.34 (s, 1H, 14c), 7.04 - 7.28 (m, 5H, Ar-H), 7.23 - 7.28 (m, 1H, Ar-H), 7.45 - 7.48 (d, *J* = 7.45, 1H, Ar-H), 7.58 - 7.65 (m, 1H, Ar-H), 8.33 (s, 1H, indole-NH). <sup>13</sup>C-NMR: 250 MHz (CDCl<sub>3</sub>): δ 23.54 (9), 24.13 (5), 44.07 (6), 50.84 (8), 55.84 (14c), 110.71 (11), 111.88 (14b), 118.73 (14), 119.52 (13), 120.99 (12), 126.24 (2), 126.4 (3), 127.92 (14a), 128.3 (1), 128.65 (4), 132.71 (9a), 133.35 (4a), 136.15 (14d), 138.04 (10a). Anal. (C<sub>19</sub>H<sub>18</sub>N<sub>2</sub> x 0.4 EtOAc): C, H, N

## **II. Experimental Procedures for the Pharmacological assays**

### **Dopamine Receptor Affinity**

#### **i) Cell culture**

D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, and D<sub>5</sub> were expressed in human embryonic kidney (HEK) cells and D<sub>4.4</sub> receptors were expressed in Chinese hamster ovary (CHO) cells. Cells were grown at 37°C under an atmosphere of 5% CO<sub>2</sub>: 95% air in HAM/F12-medium (Sigma-Aldrich) for CHO cells and Dulbecco's modified Eagles Medium Nutrient mixture F-12 Ham for HEK293 cells, both supplemented with 10% fetal bovine serum, 1 mM *L*-glutamine and 0.2 µg/ml of G 418 (all from Sigma-Aldrich).

#### **ii) Preparation of Whole-Cell-Suspension<sup>6</sup>**

Human D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub> receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85% confluence. The medium was removed and the cells were incubated with 3 ml trypsin-EDTA-solution (Sigma-Aldrich), to remove the cells from the culture dish. After incubation, cells were suspended in 6 ml medium, in order to stop the effect of trypsin-EDTA-solution. The

resulting suspension was centrifuged (483 RCF, 4°C, 4 min.). The pellet was re-suspended in 20 ml ice-cooled PBS (Phosphate-buffered saline: 137 mM NaCl, 2.7 mM, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub> and 1.4 mM KH<sub>2</sub>PO<sub>4</sub>) and centrifuged again. This procedure was repeated, the obtained pellet was suspended in 12 ml buffer (5 mM magnesium chloride, 50 mM TRIS-HCl, pH=7.4) and the resulting suspension was directly used for the radioligand binding assay.

#### **1.1.1.1. iii) Radioligand Binding Assay**

The binding studies were performed following the protocol previously described but in 96- well format.<sup>7</sup> The assays, using the whole-cell-suspension, were carried out in triplicate in a volume of 550 µl (final concentration): TRIS-Mg<sup>2+</sup>-buffer (345 µl), [<sup>3</sup>H]-ligand (50 µl), whole-cell-suspension (100 µl) and appropriate drugs (55 µl). Non-specific binding was determined using fluphenazine (100 µM) for D<sub>1</sub> and D<sub>5</sub> tests and haloperidol (10 µM) for D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> tests. The incubation was initiated by addition of the radioligand and was carried out in 96 deep well plates (Greiner bio-one, Frickenhausen) using a thermocycler (Thermocycler comfort®, Eppendorf, Wessling) at 27°C. The incubation was terminated after 90 min by rapid filtration with a PerkinElmer Mach III Harvester<sup>TM</sup> using a PerkinElmer Filtermat A, previously treated with a 0.25% polyethyleneimine-solution (Sigma-Aldrich) and washed with water. The filtermat was dried for 3 min at 400 watt using a microwave oven (MW 21, Clatronic, Kempen). The dry filtermat was placed on a filter plate (Omni filter plates<sup>TM</sup>, PerkinElmer Life Sciences) and each field of the filtermat was moistened with 50 µl Microscint 20<sup>TM</sup> scintillation cocktail. The radioactivity retained on the filters was counted using a Top Count NXT<sup>TM</sup> microplate scintillation counter (Packard, Ct., USA). For the determination of the K<sub>i</sub> values at least two independent experiments each in triplicate were performed.

The competition binding data were analyzed with GraphPad Prism<sup>TM</sup> software using nonlinear regression with sigmoidal dose response equation. Microsoft Excel<sup>TM</sup> was used to calculate the mean and the standard error of the mean. K<sub>i</sub> values were calculated from IC<sub>50</sub> values applying the equation of Cheng and Prusoff.<sup>8</sup>

## **Functional assay: Measuring intracellular $\text{Ca}^{2+}$ with a fluorescence microplate reader<sup>7,9</sup>**

### **i) Cell Culture**

Human  $\text{D}_1$  and  $\text{D}_{2\text{L}}$  receptors were stably expressed in human embryonic kidney cells (HEK293) and cultured as mentioned above.

### **ii) Preparation of Whole-Cell-Suspension**

Human  $\text{D}_1$  and  $\text{D}_{2\text{L}}$  receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85-90% confluence. The medium was removed via a suction apparatus and the cells were rinsed twice with 6 ml Krebs-HEPES buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 4.2 mM  $\text{NaHCO}_3$ , 11.7 mM D-Glucose, 1.3 mM  $\text{CaCl}_2$ , 10 mM HEPES, pH 7.4). After these two washes, cells were loaded with 3  $\mu\text{l}$  of a 0.5 M Oregon Green<sup>TM</sup> 488 BAPTA-1/AM-solution (Molecular Probes, Eugene, OR) (in DMSO) in 6 ml Krebs-HEPES buffer containing 3  $\mu\text{l}$  of a 20% Pluronic F-127-solution (Sigma Aldrich) (in DMSO) for 45 min at 37°C. After 35 min incubation, the culture dish was rapped slightly in order to remove all cells from the dish for further incubation. To this cell suspension 5ml Krebs-HEPES buffer were added again to rinse all cells from the plate. The resulting suspension was portioned into ten 1.5 ml Eppendorf caps and centrifuged at 10640 RCF for 10 seconds. The supernatant buffer was removed and the resulting ten pellets were divided into two portions à 5 pellets, each portion was suspended in 1 ml Krebs-HEPES buffer. The two suspensions were centrifuged again for 10 sec. After removing the buffer, the two pellets were combined and re-suspended in 1 ml buffer, diluted with 17 ml Krebs-HEPES buffer and plated into 96-well plates (OptiPlate HTRF-96<sup>TM</sup>, Packard, Meriden, CT; Cellstar, Tissue Culture Plate, 96W, Greiner bio-one, Frickenhausen). The microplates were kept at 37°C under an atmosphere containing 5%  $\text{CO}_2$  for 30 min before they were used for the assay.

### **iii) Calcium Assay<sup>7</sup>**

Screening for agonistic and antagonistic activity was performed using a NOVOstar microplate reader<sup>TM</sup> (BMG LabTechnologies) with a pipettor system. Agonistic activities were tested by injecting 20  $\mu$ l buffer alone as negative control, standard agonist in buffer as positive control, and rising concentrations of the test compounds in buffer, each into separate wells. Fluorescence measurement started simultaneously with the automatic injection. SKF 38393 was used as standard agonist for D<sub>1</sub> receptors and quinpirole for D<sub>2</sub> receptors (final concentration: 1  $\mu$ M).

Screening for antagonistic activities was performed by pre-incubating the cells with 20  $\mu$ L of the test compound dilutions (final concentrations: 100  $\mu$ M, 50  $\mu$ M, 10  $\mu$ M, 5  $\mu$ M, 1  $\mu$ M, 500 nM, 100 nM, 50 nM, 10 nM, 1 nM, 0,1 nM) at 37°C 30 min prior to injection of 20 $\mu$ L standard agonist per well. As standard agonists we used as described for the agonist screening SKF38393 for D<sub>1</sub> and quinpirole for D<sub>2</sub> receptors, respectively. Fluorescence measurement also started simultaneously with the automatic injection. At least two independent experiments, each carried out in four or six replications, were performed.

Fluorescence intensity was measured at 520 nm (bandwidth 25nm) for 30 s at 0.4 s intervals. Excitation wavelength was 485 nm (bandwidth 20 nm). Agonistic or antagonistic activities were assessed by a dose response curve, obtained by determination of the maximum fluorescence intensity of each data set and nonlinear regression with sigmoidal dose

### III. Table of elemental analysis for key compounds

Compound		C	H	N
<b>2</b>	found	82.37	7.35	10.03
	calc. for C <sub>19</sub> H <sub>20</sub> N <sub>2</sub>	82.57	7.29	10.14
<b>3</b>	found	79.50	7.92	8.83
	calc. for C <sub>20</sub> H <sub>22</sub> N <sub>2</sub> x 0.67 H <sub>2</sub> O	79.43	7.78	9.26
<b>4</b>	found	81.40	7.57	9.38

	$\text{C}_{20}\text{H}_{22}\text{N}_2 \times \frac{1}{4} \text{H}_2\text{O}$	81.45	7.69	9.50
<b>8</b>	found	81.48	6.13	10.59
	calc. for $\text{C}_{18}\text{H}_{16}\text{N}_2 \times \frac{1}{4} \text{C}_2\text{H}_5\text{OH}$	81.74	6.49	10.30
<b>9</b>	found	56.71	4.65	6.88
	calc. for $\text{C}_{19}\text{H}_{19}\text{IN}_2$	56.73	4.76	6.96
<b>10*</b>	found	72.10	6.64	7.58
	calc. for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2 \times 0.67 \text{EtOAc}$	72.30	7.01	7.13
<b>12</b>	found	70.50	5.85	8.30
	calc. for $\text{C}_{19}\text{H}_{18}\text{N}_2 \times \frac{1}{2} \text{CHCl}_3$	70.11	5.58	8.39
<b>13</b>	found	57.04	5.25	6.46
	calc. for $\text{C}_{20}\text{H}_{21}\text{IN}_2 \times \frac{1}{4} \text{H}_2\text{O}$	57.08	5.15	6.66
<b>15</b>	found	79.60	6.53	9.25
	calc. for $\text{C}_{19}\text{H}_{18}\text{N}_2 \times 0.4 \text{EtOAc}$	79.92	6.90	9.05
<b>16</b>	found	55.42	5.27	6.30
	calc. for $\text{C}_{20}\text{H}_{21}\text{IN}_2 \times \text{H}_2\text{O}$	55.31	5.34	6.45

\*GC/MS shows over 98% purity

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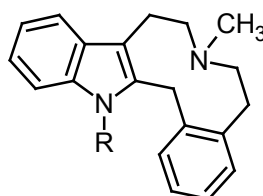
## Paper 2

### Residues at the Indole-*NH* of LE300 Modulate Affinities and Selectivities for Dopamine Receptors

Dina Robaa, Robert Kretschmer, Oliver Siol, Shams ElDin AbulAzm, ElSayed ElKhawass, Jochen Lehmann and Christoph Enzensperger

*Arch. Pharm. Chem. Life Sci.* **2011**, 344, 28-36.

**Abstract:** A series of LE300 derivatives, substituted at the indole-*NH* by a variety of residues, has been prepared and screened for the affinities for the dopamine receptors ( $D_1$ - $D_5$ ). Both the affinities and selectivities for the dopamine receptors were found to be affected by the nature of the substituent. The *N*14-methylated derivative displayed the highest affinities for all receptors. In general, the affinities decreased with increasing chain length of the *N*-alkyl chain. Different substituents partly led to altered affinity and/or selectivity profile when compared with our lead LE300, most marked was the allylated derivative which showed the highest affinity for the  $D_4$ -receptor.



R= CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>, CH<sub>2</sub>CH<sub>2</sub>F, n-C<sub>3</sub>H<sub>7</sub>, CH<sub>2</sub>-CH=CH<sub>2</sub>,  
CH<sub>2</sub>-C≡CH, COCH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>, CH<sub>2</sub>-◁, n-C<sub>5</sub>H<sub>11</sub>, n-C<sub>8</sub>H<sub>17</sub>

**Contribution:** Synthesis and characterization of all target and intermediary compounds, development of SAR and preparation of the manuscript.



## Full Paper

## Residues at the Indole-NH of LE300 Modulate Affinities and Selectivities for Dopamine Receptors

Dina Robaa<sup>1</sup>, Robert Kretschmer<sup>1</sup>, Oliver Siol<sup>2</sup>, Shams EIDin AbulAzm<sup>3</sup>, ElSayed ElKhawass<sup>3</sup>, Jochen Lehmann<sup>1,4</sup>, and Christoph Enzensperger<sup>1</sup>

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To further investigate SAR in the class of azecine-type dopamine receptor antagonists, we synthesized a series of derivatives, substituted at the indole-NH of the lead compound LE300 by different alkyl chains in addition to phenylpropyl, allyl, propargyl, and acetyl residues. The affinities of the target compounds for all human dopamine receptors (D<sub>1</sub>–D<sub>5</sub>) were investigated by radioligand binding assay and their functionality by a calcium assay. Both the affinities and selectivities for the dopamine receptors were found to be affected by the nature of the substituent. The N14-methylated derivative displayed the highest affinities for all D-receptors. In general, the affinities decreased with increasing chain length of the N-alkyl. Different substituents, partly led to altered affinity, and selectivity profile when compared with our lead LE300.

**Keywords:** azecines / dopamine receptors / indole-NH / SAR

Received: April 29, 2010; Revised: June 7, 2010; Accepted: June 11, 2010

DOI 10.1002/ardp.201000121

## Introduction

Dopamine is a key neurotransmitter in the brain, playing a regulatory role in locomotion, cognition, emotion and event prediction. Dysfunctions of the dopaminergic system have been linked to several neuropsychiatric diseases; hence dopamine receptors have long served as attractive targets for drug development [1]. Dopamine receptor antagonists, for instance, have been implemented as antipsychotics. The azecine-type dopamine receptor antagonists, based on the lead compound **1** (LE 300), represent a novel class of dopamine receptor ligands [2–4]. They were found to possess high affinities for dopamine receptors and a unique selectivity profile for the D<sub>1</sub>-like receptors.

Some previous studies have shown that substitution of the indole-NH might be advantageous; indole-NH-methylation of

the methoxylated derivative (**2**) led to increased affinities for all dopamine receptors except for D<sub>1</sub> [5]. The N14-methylated derivative of **1** was subsequently synthesized and as expected showed similar promising affinities. Hence, we synthesized a series of derivatives of **1**, substituted at the indole-NH with different alkyl chains, in order to study the effect of the substituent's length and bulk on the affinities. Moreover, N-allyl-, propargyl-, and acyl derivatives were prepared, which possess different electronic as well as steric properties compared with the alkyl groups. Finally, **1** was substituted with a phenylpropyl group, to examine the effect of both an additional aromatic group and a bulky moiety. The affinities of the prepared compounds for the human dopamine receptors (D<sub>1</sub>–D<sub>5</sub>) were investigated by radioligand binding experiments, and their functionality as agonists or antagonists was determined by a functional Calcium assay at the D<sub>1</sub>-, D<sub>2</sub>-, and D<sub>5</sub>-receptors.

## Chemistry

For the synthesis of the quinolizine **3**, we simplified previous methods to a one-step reaction, which we already adopted for

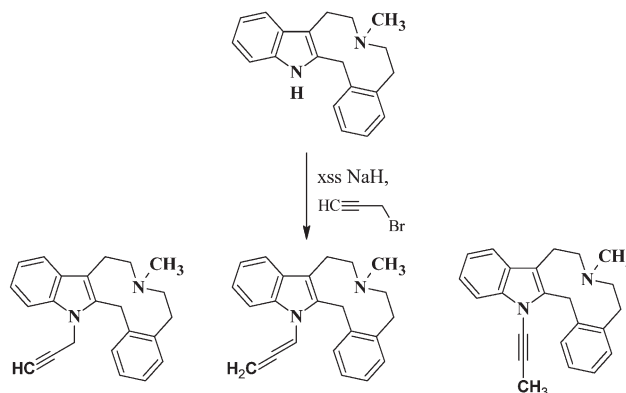
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the synthesis of analogous quinolizines [6]. Tryptamine was refluxed with 2-(2-bromoethyl)benzaldehyde in dioxane in the presence of an equimolar amount of trifluoroacetic acid. This one-pot Pictet-Spengler procedure, showed obvious advantages over the previously applied one, where tryptamine was first reacted with 1-isochromanone and the resulting amide cyclised (Bischler-Napieralski) using  $\text{POCl}_3$  followed by  $\text{NaBH}_4$  reduction [4, 7]. The obtained quinolizine was then quaternized with methyl iodide, and the quaternary salt was treated under Birch conditions with sodium in liquid ammonia to afford the indolobenzazecine **1**. Substitution of the indole-NH was achieved by the reaction of **1** with the corresponding halide derivative after deprotonation with sodium hydride in DMF. For this purpose a twofold excess of NaH and only an equimolar amount of the halide derivative were used, to ensure that no quaternization took place at the basic, central alicyclic N. The N7,N14-dimethylated compound **5** was obtained by treating the quinolizine with excess of methyl iodide in the presence of sodium hydride, which led to the simultaneous N14-alkylation and N7-quaternisation. The formed quaternary salt **4** was then cleaved under Birch conditions (Scheme 1).

However, when trying to synthesize the propargyl derivative **11**, three different products were obtained. The GC-MS spectrum showed 3 peaks of the same mass ( $m/z$  328), beside the peak of the starting material. Even when the reaction temperature was decreased to  $0^\circ\text{C}$  and the reaction time was reduced from 24 h to 2 h, a mixture of the three products in addition to the starting material was obtained. We assume that the excess of NaH led to a base-catalyzed alkyne/allene

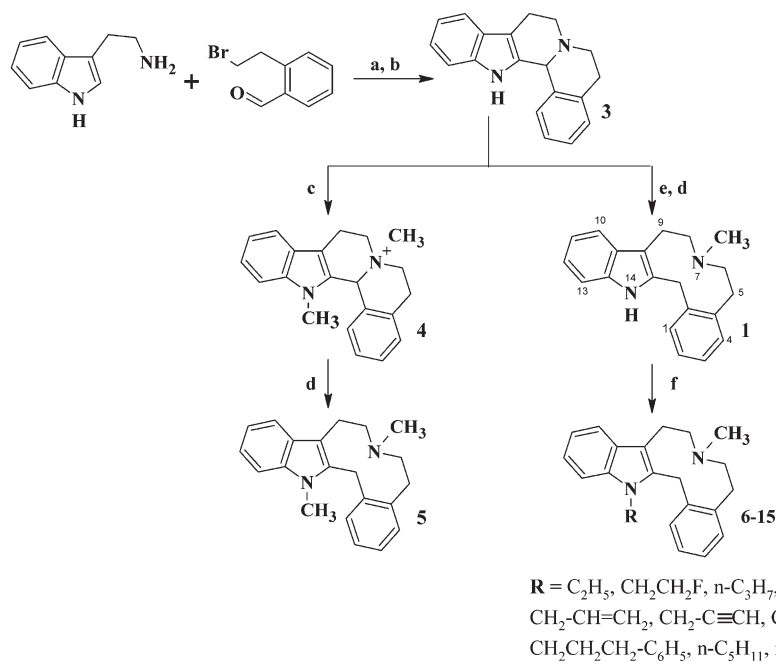


**Figure 1.** Alkyne/allene rearrangement of compound **11**.

rearrangement (Fig. 1), which has been described for acetylenes [8]. A mixture of the 2-propynyl, 1-propynyl, and allenyl derivatives was obtained, but could not be separated. The  $^{13}\text{C}$ -NMR spectrum also showed characteristic peaks for the three predicted products. Therefore, only an equimolar amount of NaH was used. This did not affect a complete reaction, hence accounting for the low yield. However it led to the propargyl derivative as a sole product.

## Pharmacology

All the target compounds were screened for their affinities for the human cloned dopamine receptor subtypes  $\text{D}_1$ ,  $\text{D}_{2\text{L}}$ ,  $\text{D}_3$ ,  $\text{D}_{4.4}$ , and  $\text{D}_5$ . These receptors were stably expressed in



**Scheme 1.** Synthesis of N14-alkylated LE300. Reagents and conditions: (a) Dioxane, TFA, reflux, 18 h; (b) NaOH; (c) NaH, THF, xss methyl iodide,  $0^\circ\text{C}$ –r.t., 24 h; (d)  $\text{Na}^0$ , liq.  $\text{NH}_3$ ,  $-40^\circ\text{C}$ , 10 min; (e) Methyl iodide, acetone, r.t., 4 h; (f) NaH, DMF, RX,  $0^\circ\text{C}$ –r.t., 24 h (warm to  $40^\circ\text{C}$  with acetyl chloride).

HEK293 or CHO cells; [ $^3\text{H}$ ]SCH 23390 and [ $^3\text{H}$ ]spiperone were used as radioligands at the D<sub>1</sub>-like and D<sub>2</sub>-like receptors, respectively.  $K_i$  values are given in nanomolar units (Tab. 1). The detailed protocol has been previously described [9].

The functionality at the D<sub>1</sub>-, D<sub>2</sub>-, and D<sub>5</sub>-receptors was tested in an intracellular calcium fluorescence assay [10], where all prepared compounds were found to possess antagonistic properties.

As some of the prepared compounds displayed interestingly high affinities for the dopamine receptors, the cytotoxicity of some selected compounds was investigated, in order to test out their applicability in an *in-vivo* assay as antipsychotics. Moreover, it was of interest to study the effect of the different substituents on the cytotoxicity of the compounds. This was tested in an MTT cell proliferation assay, the protocol has been described [11]. ( $\text{CC}_{50}$ -values are given in Table 1).

## Results and discussion

With regard to the binding profile (Table 1), all N14-alkylated derivatives showed a higher affinity for the D<sub>1</sub>-like receptors. The N14-methylated derivative **5** displayed the highest affinities among all prepared derivatives. Compared with the lead **1**, compound **5** showed a 5–15 fold increase in affinities for all D-receptor subtypes, except for D<sub>1</sub>. The ethylated derivative **6** displayed lower affinities than the methylated one (**5**), but they were nearly equal to those of **1**. It is quite evident, that the affinities decreased with increasing alkyl chain length. This was most pronounced in the N14-octyl derivative **15**, which showed the lowest affinities among all tested compounds. The only exception was the pentyl derivative **14**, which displayed lower affinities for D<sub>5</sub> than **15**; in fact it showed the highest D<sub>1</sub>/D<sub>5</sub>-selectivity (1:10).

Modification of the alkyl chains did not lead to significant alterations in the affinities for the different D-receptor. The fluoroethylated derivative **7** exhibited affinities similar to the ethylated one. The cyclopropylmethyl derivative **9** generally showed affinities similar to the N14-propyl derivative **8**, except at D<sub>5</sub>, where they were much lower.

Compared with **1**, the affinities of the N-acetylated compound **12** for the D<sub>1</sub>- and D<sub>3</sub>-receptors were lower, those for D<sub>4</sub> and D<sub>5</sub> almost equal, while a slight enhancement in the affinity for D<sub>2</sub> could be observed.

The allylated derivative **10** displayed an unexpected selectivity profile; showing the highest affinity for D<sub>4</sub>-subtype among all synthesized compounds. A similar effect was not noticed in compound **11**, where the indole-NH is substituted with a propargyl group. In spite of its bulk, the propargyl derivative exhibited rather high affinities for the D<sub>1</sub>- and the D<sub>5</sub>-receptor. This might be explained in different ways: Either the  $\pi$ -bonds enable further interactions with the receptors (like stacking) or the terminal alkyne-H

serves as H-donor. Moreover, compared with the other 3-carbon chains (N-allyl- (**10**) and N-propyl- (**8**)), the N-propargyl-derivative **11** has a straight shape and less conformational flexibility.

The derivative substituted with a phenylpropyl group (**13**) also showed significantly reduced affinities to all subtypes.

The longer alkyl chains like pentyl- (**14**) and octyl- (**15**) showed an interesting effect on the binding to the D<sub>1</sub>-family: For the D<sub>1</sub>-receptor the pentyl derivative (**14**) has a clearly higher affinity than the octyl-derivative (**15**). This is in line with all other receptor subtypes except the D<sub>5</sub> receptor, where the affinity of octyl-derivative (**15**) is higher than that of the pentyl-derivative **14**. The phenylpropyl-derivative (**13**), which might display similar space filling like **15**, has a higher affinity for the D<sub>5</sub>-receptor than the much smaller pentyl-derivative (**14**).

The fact that substitution of indole-NH with small groups (Me and Et) did not decrease the affinities, proves that the indole-NH does not play a role as H donor in the interaction with the receptor. We assume that the enhancing effect of methyl substitution on the affinities is rather caused by steric effects.

It is apparent that increased alkyl chain length goes along with increased cytotoxicity. The methylated derivative **5** exhibited a low cytotoxicity, whereas the octyl derivative **15** displayed a sixfold increase in cytotoxicity. The high affinities together with the low cytotoxicity of the methylated derivative, rendered it suitable further *in-vivo* studies, the results are reported in a separate paper.

In conclusion: To elucidate the effect of indole-NH substitution, eleven new N14-substituted benzindoloazecines were synthesized and their affinities for all dopamine receptors were determined by radioligand binding experiments. The affinities and even selectivity were seriously affected by the nature and bulk of the substituent. Interesting is also that many N14-substituted derivatives showed an increase in the affinity for D<sub>4</sub>. All in all, a clear correlation (Fig. 2) can be seen between the chain length and the affinities; an optimal binding affinity was obtained with a methyl group. Longer alkyl chains and bulky substituents were unfavorable. However, against this trend, longer alkyl chains seem to influence the binding to the D<sub>1</sub>- and the D<sub>5</sub>-receptor in opposing directions. The pentyl-derivative (**14**) has the lowest D<sub>5</sub>-affinity and longer chains seem to be better tolerated at the D<sub>5</sub>-receptor than at the D<sub>1</sub>-receptor. This effect can be further exploited in order to gain D<sub>1</sub>- or D<sub>5</sub>-selective compounds, which might be valuable tools but are not yet available [12].

Furthermore, the cytotoxicity of the synthesized derivatives increased with increasing chain length. On the whole, the N14-methylated derivative showed the most interesting affinities together with lowest cytotoxicity, which rendered it

**Table 1.** Affinities ( $K_i$ , nM) for human D<sub>1</sub>–D<sub>5</sub>-receptors, determined by radioligand binding experiments and cytotoxicity (CC<sub>50</sub>,  $\mu$ M)

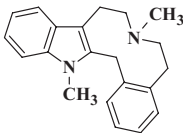
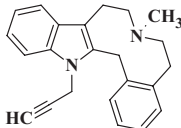
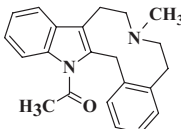
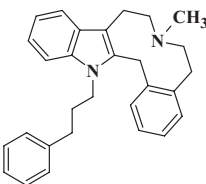
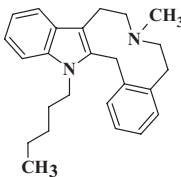
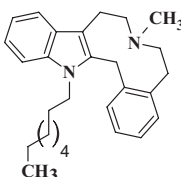
Compounds	$K_i$ , [nM]					Cytotoxicity CC <sub>50</sub> , [ $\mu$ M] <sup>c</sup>
	HEK D <sub>1</sub>	HEK D <sub>2L</sub>	HEK D <sub>3</sub>	CHO D <sub>4.4</sub>	HEK D <sub>5</sub>	
 1 (LE300)	1.9 ( $\pm$ 0.9) <sup>*</sup>	44.7 ( $\pm$ 15.8) <sup>*</sup>	40.3 ( $\pm$ 14.4) <sup>**</sup>	109 ( $\pm$ 39) <sup>*</sup>	7.5 ( $\pm$ 0.3) <sup>*</sup>	
 2	2.0( $\pm$ 1.6) <sup>**</sup>	1.7 ( $\pm$ 0.6) <sup>**</sup>	3.78 ( $\pm$ 2.4) <sup>**</sup>	21.55 ( $\pm$ 3.5) <sup>**</sup>	0.23 ( $\pm$ 0.06) <sup>**</sup>	
 5	2.0 ( $\pm$ 0.5) <sup>a</sup>	8.2 ( $\pm$ 5.8) <sup>b</sup>	7.2 ( $\pm$ 2.8) <sup>b</sup>	13.2 ( $\pm$ 3) <sup>b</sup>	0.5 ( $\pm$ 0.1) <sup>b</sup>	139.6 ( $\pm$ 22.1)
 6	3.2 ( $\pm$ 0.8) <sup>a</sup>	51.7 ( $\pm$ 12.4) <sup>a</sup>	181 ( $\pm$ 66) <sup>b</sup>	65.5 ( $\pm$ 28.9) <sup>a</sup>	7.5 ( $\pm$ 1.5) <sup>b</sup>	92.1 ( $\pm$ 7.8)
 7	12.6 ( $\pm$ 3.6) <sup>a</sup>	54 ( $\pm$ 5) <sup>b</sup>	119 ( $\pm$ 24) <sup>b</sup>	11.9	3.8 ( $\pm$ 0.8) <sup>b</sup>	
 8	22.3 ( $\pm$ 7.3) <sup>a</sup>	197 ( $\pm$ 5.5) <sup>b</sup>	307 ( $\pm$ 15) <sup>b</sup>	84 ( $\pm$ 11) <sup>a</sup>	9.8 ( $\pm$ 2.6) <sup>b</sup>	
 9	14.3 ( $\pm$ 0.2) <sup>b</sup>	241 ( $\pm$ 17) <sup>b</sup>	305 ( $\pm$ 68) <sup>b</sup>	39 ( $\pm$ 24) <sup>b</sup>	57.1 ( $\pm$ 40) <sup>a</sup>	
 10	27 ( $\pm$ 15) <sup>b</sup>	28.9 ( $\pm$ 9.3) <sup>b</sup>	99.3 ( $\pm$ 15) <sup>b</sup>	9.1 ( $\pm$ 3.6) <sup>a</sup>	5.5 ( $\pm$ 2) <sup>a</sup>	85.5 ( $\pm$ 8.2)



Table 1. (continued)

Compounds	K <sub>i</sub> , [nM]					Cytotoxicity CC <sub>50</sub> , [μM] <sup>c</sup>	
	HEK D <sub>1</sub>	HEK D <sub>2L</sub>	HEK D <sub>3</sub>	CHO D <sub>4.4</sub>	HEK D <sub>5</sub>		
	11	6.9 (±0.2) <sup>b</sup>	128 (± 45) <sup>a</sup>	411 (±81) <sup>b</sup>	36.5	1.2 (±0.3) <sup>b</sup>	
	12	22.7 (± 9.7) <sup>b</sup>	14.2 (±1.3) <sup>b</sup>	158 (± 46) <sup>b</sup>	126 (±0) <sup>b</sup>	9.9 (±1.2) <sup>b</sup>	
	13	116 (±30) <sup>a</sup>	356 (±16) <sup>b</sup>	896 (±169) <sup>a</sup>	1627 (±535) <sup>a</sup>	25.6 (±5.3) <sup>b</sup>	
	14	23.2 (±12) <sup>a</sup>	773 (±160) <sup>b</sup>	390 (±67.5) <sup>b</sup>	455 (±8.0) <sup>b</sup>	261 (±65) <sup>a</sup>	
	15	184 (±60) <sup>a</sup>	3383(±694) <sup>b</sup>	1760 (±532) <sup>a</sup>	1414 (±531) <sup>a</sup>	77.3 (±3) <sup>b</sup>	24.6 (±5.0)

\* Values taken from [3]

\*\* Values taken from [5]

<sup>a</sup>  $K_i$ -values are the means of three experiments; performed in triplicate, SEM is given in parantheses<sup>b</sup>  $K_i$ -values are the means of two; experiments; performed in triplicate  $\pm$  SEM<sup>c</sup>  $CC_{50}$  values are means of three experiments, standard deviation is given in parentheses

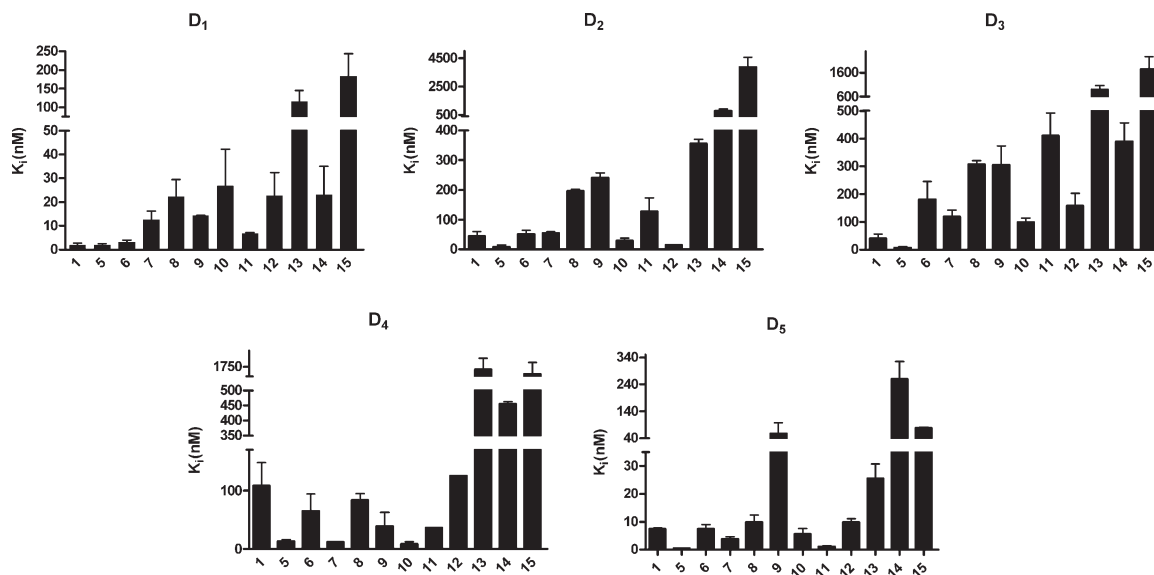
suitable for further investigation in *in-vivo* studies as a potential antipsychotic

## Experimental

### Chemistry

Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus.

<sup>1</sup>H- and <sup>13</sup>C-NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Purities of the compounds were determined by elemental analysis, performed on a Hereaus Vario EL apparatus. All values for C, H, and N were found to be within  $\pm$ 0.4. All compounds showed >95% purity.



**Figure 2.** The effect of the substituent on the affinities for the different D-receptors.

**5,6,8,9,14,14b-Hexahydroindolo[2',3':3,4]pyrido[2,1-a]-isoquinoline (3)**

A solution of tryptamine (4.2 g, 26.45 mmol), 2-(2-bromoethyl)-benzaldehyde (6.9 g, 32.4 mmol), and trifluoroacetic acid (2.99 g, 26.25 mmol) in 150 mL dioxane, kept under nitrogen, was refluxed and stirred vigorously for 18 h. The separated yellow solid was filtered off, washed with dioxane and then repeatedly with diethyl ether. The obtained solid was then alkalinized using 1 N NaOH, and the formed base was extracted with dichloromethane. Finally, the organic phase was dried over  $\text{Na}_2\text{SO}_4$ , and evaporated under reduced pressure leaving a beige solid. (4 g, yield 55.6%). M.p.: 117–119°C, (Lit. 167–168°C [7], 95–98°C and 125–127°C [13]).  $^1\text{H-NMR}$  400 MHz ( $\text{CDCl}_3$ ):  $\delta$  2.69–2.81, 2.85–3.14, 3.42–3.55 (m, 8H, 5, 6, 8, 9), 5.39 (s, 1H, 14b), 7.11–7.18 (m, 2H, 11, 12), 7.22–7.23 (d,  $J = 7$ , 1H, 4), 7.27–7.31 (m, 2H, 2, 13), 7.34–7.37 (t,  $J = 7$ , 1H, 3), 7.41–7.42 (d,  $J = 7$ , 1H, 10), 7.52–7.54 (d,  $J = 7$ , 1H, 1) 7.74 (s, 1H, NH). GC-MS:  $m/z$ : 273 (10%), 245 (6%), 230 (8%), 143 (3%), 130 (12%), 115 (17%), 89 (8%), 77 (17%), 63 (11%), 51 (16%), 42 (100%).

**7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]-benzazecine (1)**

Evaporation of the solvent yielded a creamy white powder, which was crystallized from methanol, giving white cubic crystals. Yield 69%. M.p. 90–92°C (Lit. 56–58°C [4]).  $^1\text{H-NMR}$  250 MHz ( $\text{CDCl}_3$ ):  $\delta$  2.28 (s, 3H, N-Me), 2.61–2.90 (m, 8H, 5, 6, 8, 9), 4.35 (s, 2H, 15), 7.07–7.36 (m, 7H, Ar), 7.46–7.49 (d,  $J = 7$ , 1H, Ar), 7.82 (s, 1H, NH). GC-MS:  $m/z$ : 290 (21%), 245 (22%), 230 (49%), 217 (100%), 204 (10%), 189 (15%), 177 (12%), 154 (16%), 143 (100%), 130 (48%), 115 (94%), 104 (71%), 89 (65%), 77 (71%), 63 (58%).

**7,14-Dimethyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-7-ium iodide (4)**

Yellow powder. Yield 82%. M.p. 254–255°C.  $^1\text{H-NMR}$  250 MHz ( $\text{DMSO}-d_6$ ):  $\delta$  3.25 (s, 3H,  $\text{N}^+\text{-Me}$ ), 3.78 (s, 3H, indole N-Me),

3.10–4.23 (m, 8H, 5, 6, 8, 9), 6.34 (s, 1H, 14b), 6.79–6.82 (d,  $J = 7$ , 1H, Ar), 7.10–7.16 (t,  $J = 7$ , 1H, Ar), 7.23–7.61 (m, 6H, Ar).  $^{13}\text{C-NMR}$  250 MHz ( $\text{DMSO}-d_6$ ):  $\delta$  17.7 (9), 24.3 (5), 31.1 (indole N-Me), 49.3 ( $\text{N}^+\text{-Me}$ ), 53.4 (8), 62.4 (6), 64.4 (14b), 104.0 (13), 110.7 (9a), 119.3 (1a), 120.2 (11), 123.0 (12), 125.7 (14c), 127.5 (2), 128.2 (3), 129.0 (9b), 129.3 (4), 129.4 (1), 131.4 (14a), 132.2 (4a), 138.3 (13a).

**7,14-Dimethyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]-benzazecine (5)**

Purified by column chromatography (EtOAc). Yellow powder. Yield 20%. M.p. 103–105°C.  $^1\text{H-NMR}$  400 MHz ( $\text{CDCl}_3$ ):  $\delta$  2.35 (s, 3H, N-Me), 2.45–2.48 (t,  $J = 6$ , 2H, 5), 2.70–2.73 (t,  $J = 6$ , 2H, 9), 2.76–2.81 (m, 4H, 6, 8), 3.56 (s, 3H, indole N-Me), 4.54 (s, 2H, 15), 7.04–7.08 (ddd,  $J = 5/2/0.6$ , 1H, Ar), 7.10–7.12 (dd,  $J = 7/1$ , 1H, Ar), 7.16–7.21 (m, 3H, Ar), 7.24–7.26 (d,  $J = 8$ , 1H, Ar), 7.32–7.33 (dd,  $J = 8/3$ , 1H, Ar), 7.50–7.52 (d,  $J = 8$ , 1H, Ar). GC-MS:  $m/z$ : 304 (21%), 260 (9%), 246 (53%), 232 (100%), 217 (63%), 200 (17%), 168 (10%), 157 (47%), 144 (55%), 128 (38%), 115 (88%), 104 (61%), 91 (41%), 77 (78%), 63 (34%). Anal. calcd. for  $\text{C}_{21}\text{H}_{24}\text{N}_2 \cdot 0.2 \text{ EtOAc}$ : C, 81.30%; H, 8.01%; N, 8.70%. Found: C, 81.56%; H, 8.30%; N, 8.89%.

**14-Ethyl-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (6)**

Evaporation of the solvent gave a yellow powder. Crystallized from ethanol/ $\text{H}_2\text{O}$  giving yellow crystals. Yield 56%. M.p. 103–104°C.  $^1\text{H-NMR}$  250 MHz ( $\text{CDCl}_3$ ):  $\delta$  1.01–1.07 (t,  $J = 7$ , 3H, 2'), 2.37 (s, 3H, N-Me), 2.43–2.48 (t,  $J = 6$ , 2H, 5), 2.71–2.77 (m, 6H, 6, 8, 9), 4.02–4.11 (q,  $J = 7$ , 2H, 1'), 4.58 (s, 2H, 15), 7.02–7.33 (m, 7H, Ar), 7.50–7.53 (d,  $J = 7$ , 1H, Ar).  $^{13}\text{C-NMR}$  250 MHz ( $\text{CDCl}_3$ ): 14.8 (2'), 23.3 (9), 32.8 (15), 33.23 (5), 38.2 (1'), 45.4 (N-Me), 59.0 (8), 59.4 (6), 108.8 (13), 112.8 (9a), 117.9 (10), 118.6 (11), 121.5 (12), 126.3 (3), 126.7 (2), 127.9 (9b), 131.0 (4), 131.7 (1), 134.9 (13a), 135.7 (14a), 138.1 (4a), 142.2 (15a). GC-MS:  $m/z$ : 318 (32%), 274 (9%), 260 (38%), 246 (98%), 230 (100%), 217 (76%), 202 (21%), 171 (19%), 158 (39%), 143 (44%), 130 (37%), 115 (68%), 104 (28%), 91 (33%), 77 (49%), 65

(22%). Anal. calcd. for  $C_{22}H_{26}N_2$ : C, 82.97%; H, 8.23%; N, 8.80%. Found: C, 83.05%; H, 8.44%; N, 8.70%.

**14-(2-Fluoroethyl)-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (7)**

Crystallized from Methanol then recrystallized from hexane giving white crystals. Yield 38%. M.p. 107–108°C.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ): 2.34 (s, 3H, N-Me), 2.38–2.42 (t,  $J = 6$ , 2H, 5), 2.67–2.72 (m, 6H, 6, 8, 9), 4.21 (dd, 10/4, 2H, 1'), 4.35 (dd,  $J = 6/4$ , 2H, 2'), 4.61 (s, 2H, 15), 7.03–7.33 (m, 7H, Ar), 7.52 (d,  $J = 8$ , 1H, Ar).  $^{13}C$ -NMR 250 MHz ( $CDCl_3$ ): 23.4 (9), 32.9 (15), 33.3 (5), 43.7 (d (23.3 Hz)) (1'), 45.3 (N-Me), 58.8 (8), 59.6 (6), 82.2 (d (171.7 Hz)) (2'), 108.8 (13), 113.6 (9a), 118.90 (10), 119.1 (11), 121.1 (12), 126.4 (3), 126.9 (2), 128.0 (9b), 131.2 (4), 131.9 (1), 135.3 (13a), 136.5 (14a), 137.8 (4a), 142.3 (15a). GC-MS:  $m/z$ : 336 (15%), 316 (9%), 278 (24%), 264 (51%), 230 (56%), 217 (36%), 156 (30%), 143 (33%), 128 (37%), 115 (100%), 104 (84%), 91 (44%), 78 (100%), 65 (47%). Anal. calcd. for  $C_{22}H_{25}FN_2$ : C, 78.54%; H, 7.49%; N, 8.33%. Found: 78.22%; H, 7.48%; N, 8.14%.

**7-Methyl-14-propyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (8)**

Evaporation of the solvent gave a yellow solid. Crystallized from isopropanol/ $H_2O$  giving yellow crystals. Yield 56%. M.p. 73–74°C.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ):  $\delta$  0.75–0.81 (t,  $J = 7$ , 3H, 3'), 1.43–1.52 (sextet,  $J = 7$ , 2H, 2'), 2.34 (s, 3H, N-Me), 2.42–2.47 (t,  $J = 6$ , 2H, 5), 2.69–2.73 (m, 6H, 6, 8, 9), 3.93–3.97 (t,  $J = 7$ , 2H, 1'), 4.55 (s, 2H, 15), 7.02–7.37 (m, 7H, Ar), 7.51–7.53 (d,  $J = 8$ , 1H, Ar). GC-MS:  $m/z$ : 332 (37%), 274 (23%), 260 (64%), 244 (35%), 230 (100%), 217 (67%), 202 (16%), 154 (15%), 143 (21%), 130 (24%), 115 (30%), 103 (17%), 91 (22%), 71 (30%). Anal. calcd. for  $C_{23}H_{28}N_2$ : C, 83.09%; H, 8.49%; N, 8.43%. Found: C, 83.09%; H, 8.62%; N, 8.34%.

**14-(Cyclopropylmethyl)-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (9)**

Purified by column chromatography (DCM/MeOH, 9:1), yielding a creamy white oil. Yield 63%.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ):  $\delta$  0.18–0.24 (m, 2H, 3'), 0.40–0.47 (m, 2H, 4'), 0.84–1.08 (m, 1H, 2'), 2.36 (s, 3H, N-Me), 2.48–2.53 (t,  $J = 6$ , 2H, 5), 2.71–2.78 (m, 6H, 6, 8, 9), 3.91–3.94 (d,  $J = 6$ , 2H, 1'), 4.55 (s, 2H, 15), 7.03–7.31 (m, 7H, Ar), 7.47–7.50 (d,  $J = 7$ , 1H, Ar). GC-MS:  $m/z$ : 344 (27%), 286 (13%), 272 (27%), 239 (45%), 230 (100%), 217 (60%), 202 (19%), 160 (30%), 144 (324%), 130 (44%), 115 (72%), 103 (22%), 91 (29%), 71 (34%). Anal. calcd. for  $C_{24}H_{28}N_2 \cdot 0.25 H_2O$ : C, 82.60%; H, 8.23%; N, 8.03%. Found: C, 82.48%; H, 8.32%; N, 7.91%.

**14-Allyl-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (10)**

Purified by column chromatography (EtOAc/MeOH, 2:1), yielding a colorless oil, which solidifies to a waxy white solid on standing. Yield 25%. M.p. 78–80°C.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ):  $\delta$  2.27 (s, 3H, N-Me), 2.45–2.50 (t,  $J = 6$ , 2H, 5), 2.72–2.77 (m, 6H, 6, 8, 9), 4.50 (s, 2H, 15), 4.60–4.62 (m, 2H, 1'), 4.75–4.82 (dd,  $J = 17/1$ , 1H, 3'), 4.99–5.04 (dd,  $J = 10/1$ , 1H, 3'), 5.62–5.77 (m, 1H, 2'), 7.03–7.30 (m, 7H, Ar), 7.50–7.53 (dd,  $J = 8/1$ , 1H, Ar).  $^{13}C$ -NMR 250 MHz ( $CDCl_3$ ): 23.1 (9), 32.5 (5), 33.1 (15), 45.3 (N-Me), 45.7 (1'), 58.8 (8), 59.0 (6), 109.1 (13), 113.0 (9a), 115.9 (2'), 117.8 (10), 118.8 (11), 120.8 (12), 126.3 (3), 126.7 (2), 127.8 (9b), 131.1 (4), 131.7 (1), 133.3 (3'), 135.3 (13a), 136.4 (14a), 137.8 (4a), 141.8 (15a). GC-MS:  $m/z$ : 330 (13%), 272 (22%), 258 (37%), 244 (39%), 230 (100%), 217 (60%),

202 (14%), 182 (13%), 167 (35%), 156 (17%), 144 (19%), 130 (29%), 115 (82%), 103 (34%), 91 (43%), 77 (67%), 63 (30%). Anal. calcd. for  $C_{23}H_{26}N_2 \cdot 0.25 EtOAc$ : C, 81.78%; H, 8.01%; N, 7.95%. Found: C, 81.44%; H, 8.26%; N, 8.01%.

**7-Methyl-14-prop-2-ynyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine hydrochloride (11)**

The base was purified by column chromatography (DCM/MeOH, 20:1), to separate the product from the unreacted starting material. The obtained product was converted to the HCl salt, and crystallized twice from methanol/ether yielding a white solid. Yield 11%. M.p. 171–174°C.  $^1H$ -NMR 250 MHz (MeOD):  $\delta$  2.58–2.60 (t, 1H, 3'), 2.89 (s, 3H, N-Me), 2.91–2.98 (t,  $J = 8$ , 2H, 5), 3.30–3.35 and 3.54–3.65 (m, 6H, 6, 8, 9), 4.52 (s, 2H, 1'), 4.86 (s, 2H, 15, under MeOD), 7.03–7.30 (m, 7H, Ar), 7.50–7.53 (dd,  $J = 8/1$ , 1H, Ar).  $^{13}C$ -NMR 250 MHz (MeOD): 18.3 (9), 28.1 (5), 30.1 (15), 32.2 (1'), 43.6 (N-Me), 52.8 (8), 56.5 (6), 72.2 (3'), 77.8 (2'), 109.2 (13), 109.6 (9a), 117.7 (10), 119.8 (11), 122.0 (12), 126.9 (3), 127.6 (2), 127.9 (9b), 131.8 (4), 131.4 (1), 133.4 (13a), 135.4 (14a), 135.8 (4a), 136.8 (15a). Anal. calcd. for  $C_{23}H_{24}N_2 \cdot HCl \cdot 0.25 H_2O$ : C, 74.78%; H, 6.96%; N, 7.58%; Cl, 9.60%. Found: C, 74.81%; H, 7.29%; N, 7.54%; Cl, 9.94%.

**14-Acetyl-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (12)**

Purified by column chromatography (EtOAc/MeOH, 16:5), yielding a brown oil, which solidifies to a yellowish brown solid on standing. Yield 63%. M.p. 99–100°C.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ):  $\delta$  2.28 (s, 3H, N-Me), 2.40–2.44 (t,  $J = 6$ , 2H, 5), 2.62–2.73 (m, 9H, 6, 8, 9, COMe), 4.75 (s, 2H, 15), 7.00–7.46 (m, 7H, Ar), 7.78–7.81 (dd,  $J = 8/2$ , 1H, Ar).  $^{13}C$ -NMR 250 MHz ( $CDCl_3$ ): 23.9 (9), 27.6 (COMe), 33.6 (5), 33.7 (15), 45.8 (N-Me), 58.3 (8), 60.5 (6), 114.3 (13), 118.3 (10), 121.2 (9a), 122.6 (11), 123.8 (12), 126.1 (3), 126.5 (2), 130.3 (9b), 131.1 (4), 131.7 (1), 135.9 (13a), 136.9 (14a), 138.1 (4a), 141.4 (15a), 170.5 (CO). GC-MS:  $m/z$ : 332 (12%), 289 (11%), 246 (39%), 230 (199%), 217 (91%), 202 (14%), 170 (16%), 160 (74%), 144 (31%), 130 (42%), 115 (56%), 103 (21%), 91 (24%), 77 (41%), 63 (22%). Anal. calcd. for  $C_{22}H_{24}N_2O \cdot 0.25 EtOAc$ : C, 77.93%; H, 7.39%; N, 7.90%. Found: C, 77.98%; H, 7.49%; N, 7.95%.

**7-Methyl-14-(3-phenylpropyl)-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (13)**

The base was converted to the HCl salt, and the aqueous solution was washed twice with EtOAc. The aqueous solution was then alkalized, and the formed base was extracted with chloroform. Evaporation of the solvent gave a brown oil that did not crystallize. Yield 57%.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ):  $\delta$  1.70–1.81 (q,  $J = 8$ , 2H, indole  $N-CH_2CH_2CH_2Ph$ ), 2.35 (s, 3H, N-Me), 2.38–2.52 (m, 4H, 5, indole  $N-CH_2CH_2CH_2Ph$ ), 2.68–2.76 (m, 6H, 6, 8, 9), 3.95–4.02 (t,  $J = 8$ , 2H, indole  $N-CH_2CH_2CH_2Ph$ ), 4.53 (s, 2H, 15), 7.03–7.32 (m, 12H, Ar), 7.50–7.53 (d,  $J = 7$ , 1H, Ar). GC-MS:  $m/z$ : 408 (3%), 244 (4%), 230 (8%), 217 (6%), 143 (9%), 128 (7%), 117 (17%), 104 (13%), 91 (100%), 77 (23%), 65 (22%). Anal. calcd. for  $C_{29}H_{32}N_2 \cdot 0.75 H_2O$ : C, 82.52%; H, 8.00%; N, 6.64%. Found: C, 82.88%; H, 8.30%; N, 6.46%.

**7-Methyl-14-pentyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (14)**

Purified by column chromatography (DCM/MeOH, 9.5:0.5), yielding creamy white oil. Yield 41%.  $^1H$ -NMR 250 MHz ( $CDCl_3$ ):  $\delta$

0.81–0.87 (t,  $J = 7$ , 3H, 5'), 1.41–1.49 (m, 6H, 2'–4'), 2.37 (s, 3H, N-Me), 2.44–2.48 (t,  $J = 6$ , 2H, 5), 2.72–2.77 (m, 6H, 6, 8, 9), 3.92–3.99 (t,  $J = 8$ , 2H, 1'), 4.56 (s, 2H, 15), 7.03–7.33 (m, 7H, Ar), 7.50–7.53 (d,  $J = 8$ , 1H, Ar).  $^{13}\text{C}$ -NMR 250 MHz ( $\text{CDCl}_3$ ): 13.9 (5'), 22.4 (4'), 23.2 (9), 29.1 (3'), 29.6 (2'), 32.8 (5), 33.1 (15), 43.8 (1'), 45.2 (N-Me), 58.9 (8), 59.6 (6), 109.0 (13), 112.7 (9a), 117.8 (10), 118.5 (11), 120.6 (12), 126.3 (3), 126.7 (2), 127.7 (9b), 131.0 (4), 131.7 (1), 135.1 (13a), 136.1 (14a), 138.0 (4a), 142.0 (15a). GC-MS:  $m/z$ : 360 (13%), 302 (10%), 288 (22%), 255 (50%), 244 (32%), 230 (100%), 217 (69%), 202 (19%), 160 (22%), 144 (48%), 130 (63%), 115 (90%), 105 (40%), 91 (47%), 71 (47%). Anal. calcd. for  $\text{C}_{25}\text{H}_{32}\text{N}_2 \cdot \text{HCl} \cdot 0.75 \text{H}_2\text{O}$ : C, 73.15%; H, 8.47%; N, 6.82%. Found: C, 72.90%; H, 8.74%; N, 6.64%.

### 7-Methyl-14-octyl-6,7,8,9,14,15-hexahydro-5H-indolo-[3,2-f][3]benzazecine (15)

Evaporation of the solvent gave a white oily substance, which did not crystallize. Yield 81%.  $^1\text{H}$ -NMR 400 MHz ( $\text{CDCl}_3$ ):  $\delta$  0.90–0.94 (t,  $J = 7$ , 3H, 8'), 1.20–1.45 (m, 12 H, 2'–7'), 2.40 (s, 3H, N-Me), 2.45–2.48 (t,  $J = 6$ , 2H, 5), 2.70–2.73 (t,  $J = 6$ , 2H, 9), 2.76–2.81 (m, 4H, 6, 8), 3.56 (s, 2H, 1'), 4.58 (s, 2H, 15), 7.05–7.21 (m, 5H, Ar), 7.26–7.28 (d,  $J = 8$ , 1H, Ar), 7.31–7.33 (dd,  $J = 8/2$ , 1H, Ar), 7.51–7.53 (d,  $J = 8$ , 1H, Ar).  $^{13}\text{C}$ -NMR 400 MHz ( $\text{CDCl}_3$ ): 14.1 (8'), 22.6 (7'), 23.4 (9), 27.0 (3'), 29.2 (5'), 29.3 (2'), 30.0 (4'), 31.8 (6'), 32.9 (5), 33.4 (15), 43.8 (1'), 45.3 (N-Me), 59.1 (8), 59.6 (6), 109.0 (13), 112.7 (9a), 117.8 (10), 118.5 (11), 120.6 (12), 126.3 (3), 126.7 (2), 127.8 (9b), 131.0 (4), 131.7 (1), 135.2 (13a), 136.1 (14a), 138.1 (4a), 142.3 (15a). GC-MS:  $m/z$ : 402 (23%), 344 (14%), 297 (28%), 260 (9%), 246 (38%), 230 (73%), 217 (37%), 200 (6%), 171 (7%), 158 (26%), 144 (33%), 129 (30%), 115 (64%), 104 (41%), 91 (32%), 77 (100%). Anal. calcd. for  $\text{C}_{28}\text{H}_{38}\text{N}_2$ : C, 83.53%, H, 9.51%, N, 6.96%. Found: C, 83.38%, H, 9.18%, N, 6.46%.

## Pharmacology

### Radioligand binding assay

The binding studies were performed following the protocol previously described but in 96-well format [3]. The assays, using the whole-cell-suspension, were carried out in triplicate in a volume of 550  $\mu\text{L}$  (final concentration): TRIS- $\text{Mg}^{2+}$ -buffer (345  $\mu\text{L}$ ), [ $^3\text{H}$ ]-ligand (50  $\mu\text{L}$ ), whole-cell-suspension (100  $\mu\text{L}$ ) and appropriate drugs (55  $\mu\text{L}$ ). Non-specific binding was determined using fluphenazine (100  $\mu\text{M}$ ) for  $\text{D}_1$ - and  $\text{D}_5$ -tests and haloperidol (10  $\mu\text{M}$ ) for  $\text{D}_2$ -,  $\text{D}_3$ -, and  $\text{D}_4$ -tests. The incubation was initiated by addition of the radioligand and was carried out in 96 deep well plates (Greiner bio-one, Frickenhausen) using a thermocycler (Thermocycler comfort<sup>®</sup>, Eppendorf, Wessling) at 27°C. The incubation was terminated after 90 min by rapid filtration with a PerkinElmer Mach III Harvester<sup>™</sup> using a PerkinElmer Filtermat A, previously treated with a 0.25% polyethyleneimine-solution (Sigma-Aldrich) and washed with water. The filtermat was dried for 3 min at 400 W using a microwave oven (MW 21, Clatronic, Kempen). The dry filtermat was placed on a filter plate (Omni filter plates<sup>™</sup>, PerkinElmer Life Sciences) and each field of the

filtermat was moistened with 50  $\mu\text{L}$  Microscint 20<sup>™</sup> scintillation cocktail. The radioactivity retained on the filters was counted using a Top Count NXT<sup>™</sup> microplate scintillation counter (Packard, Ct., USA). For the determination of the  $K_i$ -values at least two independent experiments each in triplicate were performed.

The competition binding data were analyzed with GraphPad Prism<sup>™</sup> software using nonlinear regression with sigmoidal dose response equation. Microsoft Excel<sup>™</sup> was used to calculate the mean and the standard error of the mean.  $K_i$ -values were calculated from  $\text{IC}_{50}$ -values applying the equation of Cheng and Prusoff [14].

### Functional assay

Measuring intracellular  $\text{Ca}^{2+}$  with a fluorescence microplate reader [3, 10]. Human  $\text{D}_1$ - and  $\text{D}_{2L}$ -receptors were stably expressed in human embryonic kidney cells (HEK293) and cultured as mentioned above. Human  $\text{D}_1$ - and  $\text{D}_{2L}$ -receptor cell lines were grown on T 175-culture dishes (Greiner bio-one, Frickenhausen) to 85–90% confluence. The medium was removed via a suction apparatus and the cells were rinsed twice with 6 mL Krebs-HEPES buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 4.2 mM  $\text{NaHCO}_3$ , 11.7 mM D-Glucose, 1.3 mM  $\text{CaCl}_2$ , 10 mM HEPES, pH 7.4). After these two washes, cells were loaded with 3  $\mu\text{L}$  of a 0.5 M Oregon Green<sup>™</sup> 488 BAPTA-1/AM-solution (Molecular Probes, Eugene, OR) (in DMSO) in 6 mL Krebs-HEPES buffer containing 3  $\mu\text{L}$  of a 20% pluronic F-127-solution (Sigma Aldrich) (in DMSO) for 45 min at 37°C. After 35 min incubation, the culture dish was rapped slightly in order to remove all cells from the dish for further incubation. To this cell suspension 5 mL Krebs-HEPES buffer were added again to rinse all cells from the plate. The resulting suspension was portioned into ten 1.5 mL Eppendorf caps and centrifuged at 10640 RCF for 10 s. The supernatant buffer was removed and the resulting ten pellets were divided into two portions à 5 pellets, each portion was suspended in 1 mL Krebs-HEPES buffer. The two suspensions were centrifuged again for 10 s. After removing the buffer, the two pellets were combined and re-suspended in 1 mL buffer, diluted with 17 mL Krebs-HEPES buffer and plated into 96-well plates (OptiPlate HTRF-96<sup>™</sup>, Packard, Meriden, CT; Cellstar, Tissue Culture Plate, 96W, Greiner bio-one, Frickenhausen). The microplates were kept at 37°C under an atmosphere containing 5%  $\text{CO}_2$  for 30 min before they were used for the assay.

Screening for agonistic and antagonistic activity was performed using a NOVostar microplate reader<sup>™</sup> (BMG LabTechnologies) with a pipettor system. Agonistic activities were tested by injecting 20  $\mu\text{L}$  buffer alone as negative control, standard agonist in buffer as positive control, and rising concentrations of the test compounds

in buffer, each into separate wells. Fluorescence measurement started simultaneously with the automatic injection. SKF 38393 was used as standard agonist for D<sub>1</sub>-receptors and quinpirole for D<sub>2</sub>-receptors (final concentration: 1  $\mu$ M).

Screening for antagonistic activities was performed by pre-incubating the cells with 20  $\mu$ L of the test compound dilutions (final concentrations: 100  $\mu$ M, 50  $\mu$ M, 10  $\mu$ M, 5  $\mu$ M, 1  $\mu$ M, 500 nM, 100 nM, 50 nM, 10 nM, 1 nM, 0.1 nM) at 37°C 30 min prior to injection of 20  $\mu$ L standard agonist per well. As standard agonists, we used as described for the agonist screening SKF38393 for D<sub>1</sub>- and quinpirole for D<sub>2</sub>-receptors, respectively. Fluorescence measurement also started simultaneously with the automatic injection. At least two independent experiments, each carried out in four or six replications, were performed.

### MTT-test

U87-MG glia cells (ATCC HTB-14) were cultured at 37°C, and 5% CO<sub>2</sub> in DMEM (PAA, E15-883) + 10% FCS (Thermo Scientific HyClone). In 96-well plates 15 000 cells were dispersed in 200  $\mu$ L into each well. After 24 h the medium was replaced by DMEM + FCS containing the test compound dissolved in finally 0.25% DMSO. To minimize edge-effects as mentioned by Rasmussen [15] each concentration of the compound was measured in 6 wells distributed across the 96-well plate and only the inner 6  $\times$  10 wells of the plate were analyzed. The outer wells were filled with 200  $\mu$ L PBS-buffer. As positive control, 6 wells with cells and medium containing 0.25% DMSO were analyzed. After 24 h of incubation, the medium was removed and 100  $\mu$ L MTT (Fluka), dissolved in phenol red-free DMEM (without FCS) at 0.5 mg/mL, were added to each well. The plates were incubated for 4 h. Cells were killed and formazan crystals were solubilized by addition of 100  $\mu$ L of 20% sodium dodecylsulfate (SDS) in H<sub>2</sub>O followed by incubation overnight at 37°C. Optical density was measured at 544 nm using a microplate reader (Galaxy FluoStar, BMG Labtechnologies) with background subtraction (compounds in the same preparation without cells). Cytotoxicity values were calculated as percentage of positive control (= 100%). CC<sub>50</sub>-values were calculated with

Graph Pad Prism <sup>TM</sup> 3.0. All experiments were repeated at least three times.

We thank Bärbel Schmalwasser, Petra Wiecha, and Heidi Traber for skillful technical assistance in performing the pharmacological assays. Dina Robaa thanks the Egyptian Cultural Affairs and Mission Section for her stipend. Furthermore we would like to thank the DFG for the financial support of the project (EN 875/1-1). Robert Kretschmer thanks the Hans-Böckler-Stiftung for a scholarship.

The authors have declared no conflict of interest.

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## Paper 3

### **Molecular Combination of the Dopamine and Serotonin Scaffolds Yields Novel Antipsychotic Drug Candidates – Characterization by *in vivo* Experiments**

*Maria Schulze, Oliver Siol, Dina Robaa, Franziska K. U. Mueller, Christian Fleck, and Jochen Lehmann*

*Arzneimittelforschung; manuscript submitted*

**Abstract:** In this work the potential antipsychotic activity of some azecine derivatives was characterized by radioligand binding experiments and conclusively confirmed *in vivo* (rats) by applying a conditioned avoidance response (CAR) model. Furthermore, the compounds were tested *in vivo* for the development of catalepsy, which is a predictive parameter for extra-pyramidal side-effects caused by many antipsychotics. The antipsychotic activities of the tested azecines were comparable with those of haloperidol and risperidone, but revealed a two to five times better therapeutic range with regard to catalepsy. Preliminary tests for oral bioavailability also revealed promising results for the indole-*N* methylated LE300 (LE300ME).

**Contribution:** Synthesis of LE300 and the methylated LE300 derivative (LE300ME), revision of the manuscript.





# Molecular Combination of the Dopamine and Serotonin Scaffolds Yield Novel Antipsychotic Drug Candidates – Characterization by *in vivo* Experiments

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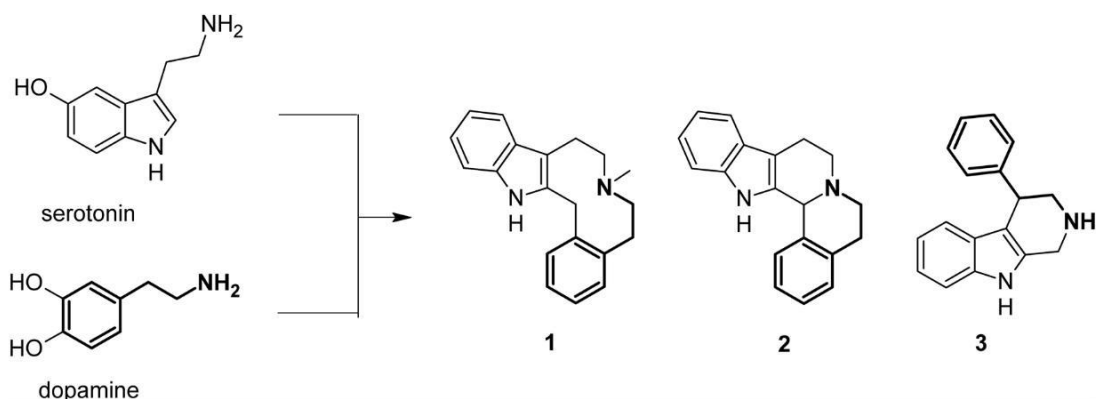
**Abstract.** Serotonin and dopamine play an important role in the aetiology of schizophrenia. Combination of the structural scaffolds of both neurotransmitters in a single molecule led to aromatic annelated [d,g]azecines, which showed to be nanomolar to subnanomolar dopamine D<sub>1</sub>-D<sub>5</sub> receptor antagonists. In this work the potential antipsychotic activity of some azecine derivatives was characterized by radioligand binding experiments and conclusively confirmed *in vivo* (rats) by applying a conditioned avoidance model. Furthermore, the compounds were tested *in vivo* for the development of catalepsy, which is considered to be improved by 5-HT<sub>2A</sub> antagonism and which is a predictive parameter for extra-pyramidal side-effects caused by many antipsychotics. The investigated azecines displayed low cytotoxicity and affinities at human dopamine D<sub>1</sub>-D<sub>5</sub> and serotonin 5-HT<sub>2A</sub> receptors in a nanomolar range. Their antipsychotic activities in the rat model were comparable with those of haloperidol and risperidone, but revealed a two to five times better therapeutic range with regard to catalepsy. Preliminary tests for oral bioavailability also revealed promising results for this new class of potential antipsychotic compounds. In conclusion, aromatic annelated [d,g]azecines represent a novel class of substances with interesting qualities as potential atypical antipsychotics.

## 1. Introduction

Due to a lack of efficacy and severe side-effects of the commonly used drugs, the treatment of schizophrenia is still a problem. In the last decades, the focus of scientific research on antipsychotic drugs moved step by step towards the use of non-selective compounds with both dopamine and serotonin receptor affinities. Classical antipsychotics are predominantly dopamine D<sub>2</sub> receptor antagonists. They are very potent in treating positive symptoms, which are mainly caused by dopamine hyperfunction in the mesolimbic system. On the other hand, these drugs are not very useful to treat the negative symptoms and often produce intolerable extra-pyramidal side-effects, which are both, at least partially, caused by a dopamine hypofunction in some

brain regions (Knable et al., 1997; Abi-Dargham and Laruelle, 2005). With the development of atypical antipsychotics, the hypotheses of an influence of serotonin on schizophrenia arose (Moller, 1999). It is not clarified yet, what influence serotonin has on the symptoms. One main hypothesis is an indirect connection of serotonin 2A receptor (5-HT<sub>2A</sub>) antagonism on dopamine release in the mesocortex, which acts beneficially on the negative symptoms but may also affect extra-pyramidal side-effects caused by dopamine D<sub>2</sub> antagonists (Ichikawa and Meltzer, 1999).

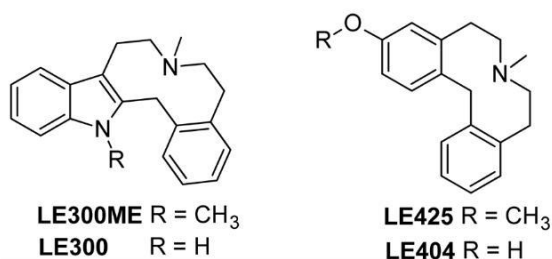
This hypothesis is encouraged by the benefit of 5-HT<sub>2A</sub> antagonists in *in vivo* studies (Meltzer et al., 1989; Kehne et al., 1996; Kleven et al., 1996; Prinssen et al., 2002).



**Figure 1** Lead structure development starting from the structural scaffolds of dopamine and serotonin.

We have been interested to combine the basic structures of both neurotransmitters into a single molecule, hopefully achieving simultaneously high affinities for both, dopamine and serotonin receptors and thus go for new potential antipsychotic drugs with better therapeutic indices concerning positive symptoms and extra-pyramidal side-effects. Furthermore a beneficial influence on negative symptoms was supposed to be achieved.

**LE300** (compound **1**), a benzindolozecine (Figure 1), has been previously identified by us as a highly active dopamine receptor antagonist (nanomolar to subnanomolar  $K_i$ -values), whereas compounds **2** and **3** showed no affinities for dopamine receptors up to 10  $\mu$ M (Witt et al., 2000). Lead structure optimization of aromatic annelated azecines revealed as most favourable structural features an indole or 3-substituted benzene as one of the aromatic moieties and a methyl function at the basic nitrogen (Witt et al., 2000; Kassack et al., 2002; Mohr et al., 2006). Based on this we selected four azecine derivatives for further investigations (Figure 2).



**Figure 2** Compounds used in this study for *in vivo* investigations.

In this present study we determined the affinities of the selected azecines not only for the dopamine D<sub>1</sub>-D<sub>5</sub> but also for the serotonin 5-HT<sub>2A</sub> receptors and, with regard to *in vivo* application, tested their toxicity on cultured human glia cells. Based on the *in vitro* results we performed *in vivo* tests using rats with the above mentioned compounds **LE300**, **LE300ME**, **LE404** and **LE425** (Figure 2). It was examined, whether the high *in vitro* binding affinities of the azecines at the different dopamine and serotonin receptors result in high potencies in an animal model for positive symptoms. For this purpose, inhibition of conditioned avoidance response (CAR) was used, which is considered a reliable test system for the identification of potential neuroleptics (Arnt, 1982; Wadenberg and Hicks, 1999). Catalepsy tests in rats were performed in order to measure the influence of the *in vitro* determined 5-HT<sub>2A</sub> receptor affinities on extra-pyramidal side-effects *in vivo*, caused by dopamine D<sub>2</sub> antagonism. In general, we wanted to estimate *in vivo*, whether the molecular combination of dopamine and serotonin scaffolds might yield in new and more advantageous antipsychotic drugs.

## 2. Materials and methods

### 2.1. Substances

**Haloperidol** (Sigma-Aldrich, Germany), **Risperidone** (Promochem, Germany), **LE404** (7-methyl-5,6,7,8,9,14-hexahydrodibenzo[*d,g*]azecine-3-ol) (Hoefgen et al., 2006), **LE425** (3-methoxy-7-methyl-5,6,7,8,9,14-hexahydrodibenzo[*d,g*]azecine) (Hoefgen et al., 2006), **LE300** (7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benz-azecine) (Witt et al., 2000) were available in the lab from previous work.

**LE300ME** (7,14-Dimethyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine) is a new compound, prepared from 5,6,8,9,14,14b-hexahydroindolo-[2',3',3,4]pyrido[2,1-*a*]isoquinoline (Robaa et al., 2011). Chemical characterisation of this new substance is given in the supporting information.

### 2.2. *In vitro* tests

#### 2.2.1. Radioligand binding and functional calcium assay

Radioligand binding experiments and a functional calcium assay were performed as described previously (Schulze et al., 2009). In addition to the human dopamine receptor subtypes D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, D<sub>4.4</sub> and D<sub>5</sub> we established a radioligand binding assay for human cloned 5-HT<sub>2A</sub> receptors. Human 5-HT<sub>2A</sub>, D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, and D<sub>5</sub> receptors were stably expressed in human embryonic kidney cells (HEK293) and D<sub>4.4</sub> receptors in chinese hamster ovary cells (CHO), respectively. A serotonin 5-HT<sub>2A</sub> expressing cell line (HEK293) was kindly provided by M. U. Kassack (University of Düsseldorf, Germany).

#### Cell culture

Cells were grown at 37°C under an atmosphere of 5% CO<sub>2</sub>: 95% air in HAM/F12-medium (Sigma-Aldrich, Germany) for CHO cells and Dulbecco's Modified Eagles Medium Nutrient Mixture F-12 Ham (Sigma-Aldrich, Germany) for HEK293 cells, each supplemented with 10% foetal bovine serum, 1mM L-glutamine and 0.2  $\mu$ g/mL of G 418 (all from Sigma-Aldrich, Germany).

#### Radioligand binding experiments

Radioligand binding studies were performed using a whole cell suspension in tris-HCl buffer (pH 7.4). [<sup>3</sup>H]SCH23390 (Amersham, UK), was used as radioligand for the D<sub>1</sub> and D<sub>5</sub> receptor binding experiments, and [<sup>3</sup>H]spiperone (Amersham, UK) for 5-HT<sub>2A</sub>, D<sub>2L</sub>, D<sub>3</sub> and D<sub>4.4</sub> receptors. Non-specific binding was determined using fluphenazine (Sigma-Aldrich, Germany) (100  $\mu$ M) for D<sub>1</sub>, D<sub>5</sub> and 5-HT<sub>2A</sub> receptors and haloperidol (Sigma-Aldrich, Germany) (10  $\mu$ M) for D<sub>2L</sub>, D<sub>3</sub>, and D<sub>4.4</sub> experiments. The test compound (dilutions for final concentrations between 100  $\mu$ M and 1 pM) and the radioligand were added to the cell suspension and the mixture was incubated at 27 °C for 90 min using a thermocycler (Thermocycler comfort®, Eppendorf, Germany). Experiments were carried out in 96 well format (Greiner bio-one,

Germany) in a final volume of 550  $\mu$ L per well. For determining the  $K_i$  values, at least two independent experiments each in triplicate were performed. The competition binding data were analyzed with *Graph Pad Prism*<sup>TM</sup> 3.0 software using nonlinear regression with sigmoidal dose response equation.  $K_i$  values were calculated applying the equation of Cheng and Prusoff (1973).

#### *Functional calcium assay*

Screening for agonistic and antagonist activity was performed in an intracellular calcium assay using a NOVOstar microplate reader<sup>TM</sup> (BMG Lab Technologies, Germany) with a pipettor system. Agonistic activities were tested by injecting 20  $\mu$ L buffer alone as negative control, standard agonist in buffer as positive control (final concentration: 1  $\mu$ M), and test compounds in buffer in rising concentrations, respectively, each into separate wells. Screening for antagonist activities was performed by pre-incubating the cells with 20  $\mu$ L of the test compound dilutions (final concentrations: 100  $\mu$ M, 50  $\mu$ M, 10  $\mu$ M, 5  $\mu$ M, 1  $\mu$ M, 500 nM, 100 nM, 50 nM, 10 nM, 1 nM, 0.1 nM) at 37°C 30 min prior to injection of 20  $\mu$ L standard agonist per well. Fluorescence measurement started simultaneously to the automatic injection. SKF38393 (Sigma-Aldrich, Germany) was used as standard agonist for D<sub>1</sub> and D<sub>5</sub> receptors and quinpirole (Sigma-Aldrich, Germany) for D<sub>2L</sub> receptors. At least two independent experiments, each in four or six replications, were performed. Fluorescence intensity was measured at 520 nm (bandwidth 25 nm) for 30 s at 0.4 s intervals. Excitation wavelength was 485 nm (bandwidth 20 nm). Agonistic or antagonist activities were assessed by a dose response curve, obtained by determination of the maximum fluorescence intensity of each data set and nonlinear regression with sigmoidal dose response equation using *Graph Pad Prism*<sup>TM</sup> 3.0.

#### *2.2.2. Cytotoxicity - MTT-test*

U87-MG glia cells (HTB-14; ATCC, USA) were cultured at 37 °C, and 5% CO<sub>2</sub> in Dulbecco's Modified Eagles Medium (DMEM) (PAA, Austria) + 10% Foetal calf serum (FCS) (Thermo Scientific HyClone, UK). In 96 well plates 15,000 cells were dispersed in 200  $\mu$ L into each well. After 24 hours the medium was replaced by DMEM + FCS containing the test compound solved in 0.25% DMSO (final concentration). To minimize edge-effects as mentioned by Rasmussen (2002), each concentration of each compound was measured in 6 wells distributed across the 96 well plate and only the inner 6x10 wells of the plate were analyzed. The outer wells were filled with 200  $\mu$ L PBS-buffer. As positive control, 6 wells with cells and medium containing 0.25% DMSO were analyzed. After 24 hours of incubation, the medium was removed and 100  $\mu$ L MTT (Fluka, Germany), dissolved in phenol red-free DMEM (without FCS) at 0.5 mg/mL, were added to

each well. The plates were incubated for 4 hours. By addition of 100  $\mu$ L of 20% sodium dodecyl sulphate (SDS), followed by incubation overnight at 37°C, all cells were killed and formazan crystals were solubilised. Optical density was measured at 544 nm using a microplate reader (Galaxy FluoStar, BMG Lab technologies) with background subtraction (compounds in the same preparation without cells). Cytotoxicity values were calculated as percentage of positive control (= 100%). CC<sub>50</sub> values (EC<sub>50</sub> equivalents of cytotoxicity) were determined with *Graph Pad Prism*<sup>TM</sup> 3.0. All experiments were repeated at least three times independently.

### *2.3. In vivo tests*

#### *2.3.1. Animals*

Female Han-Wistar rats, aged 8 weeks and weighing between 120 and 160 g at the beginning of the experiments, were used for the pole jump experiments and the locomotor activity tests. For the catalepsy test rats aged 4-8 months were used, weighing between 200 and 280 g. Animals were kept under controlled laboratory conditions (dark-light cycle 12:12 h, temperature 23±2°C, air humidity 55-60%). They were housed in groups of four animals with free access to commercial food (Altromin 1326®, Altromin Lage, Germany) and tap water. The permission of the animal protection commission of the State of Thuringia was given.

#### *2.3.2. Administration of Substances*

**Haloperidol**, **LE404**, **LE425**, **LE300** and **LE300ME** were converted into hydrochlorides and dissolved in isotonic saline solution. **Risperidone** was used as free base, dissolved in isotonic saline solution with two drops of Tween 80. The pH was adjusted to 5 - 7.

In all experiments, except the oral bioavailability tests, 1 mL/100 g<sup>-1</sup> body weight was injected intraperitoneally. Pre-treatment time for **haloperidol** and the new test compounds **LE400**, **LE425**, **LE300** and **LE300ME** was 30 min. According to literature pre-treatment time for **risperidone** was 60 min for the pole jump experiment (Becker and Grecksch, 2008). The control groups received saline solution only.

For oral bioavailability, examined in the pole jump test, **LE404** and **LE300ME** were administered by oral gavage. Four rats were treated with 2.5 mg/kg body weight of the substances, dissolved in saline solution (0.5 mL/200 g body weight). Further investigations on **LE404** were performed on 13 animals (5 mg/kg on 8 rats, 20 mg/kg on 5 rats).

#### *2.3.3. Testing*

After a short handling period in which the rats were in close contact with the laboratory staff, at an age of 30 days, all rats were habituated to the experimental equipment and the operator 10 min per day over 10 days. Trainings and experiments were performed between 7:00 a.m. and 3:00 p.m.

#### 2.3.3.1. Locomotor activity

Horizontal and vertical movements of the rats (n=8 per dose) were observed in an open field box (50 cm x 50 cm x 50 cm) over 5 min. The bottom of the box was divided into 25 equal quarters. Rats were placed in the middle of the box. Line crossing, rearing on the edge /in the middle, grooming and defecation was counted individually over the whole time. The illumination in the test room was stable at 30 lx. The open field box was cleaned with Descosept AF (Schumacher, Melsungen, Germany) and wiped after each test. Dose response curves were generated with *Graph Pad Prism*<sup>TM</sup> 3.0.

#### 2.3.3.2. Catalepsy

Catalepsy was measured in two different test systems: the cross-leg-position test (CLP) and the bar test (Kleven et al., 1996) with 7 rats per dose. In the bar test, 30 and 90 min after drug administration, animals were placed with their forepaws on a wooden bar, which was 10 cm high. The time was measured, during which the rat remained in this position. Immediately after the bar test, the rats were exposed to the CLP test, where one of the hind limbs was placed over the forepaw and time was measured during which the rat remained immobile. Rats, which stayed in the awkward position longer than 90 s were estimated as cataleptic. Dose response curves and  $ED_{50}$  values could be obtained by plotting the amount of cataleptic rats against the individual dose by *Graph Pad Prism*<sup>TM</sup> 3.0.

#### 2.3.3.3. Pole Jump

Rats were entrained daily to jump onto a pole when a white noise signal (1000 Hz, 50 dB, conditioned stimulus, CS) was presented, in order to avoid an electric foot-shock (0.2 mA, unconditioned stimulus, UCS), which was given additionally to the noise after 4 s. Maximum trial duration was 20 s. Animals were exposed to 10 trials every day until they showed stable conditioned avoidance response (CAR). Stable CAR was defined by at least eight out of ten jumps within 4s after presentation of the white noise. Only rats with at least 6 days of training and a stable CAR over the last two days were used for the experiments.

Rats were applied to the experiment with different concentrations of one substance in randomized order. Between the experiments each rat had a seven day wash out period including training every day.

Drug tests consisted of three steps according to literature (Becker and Grecksch, 2008). First, the stable CAR of each rat was verified within 5 trials with conditioned and unconditioned stimulus as described above. Only rats which jumped in four out of five trials within 4 s (stable CAR) were used for the further experiment.

After administration of the test compounds and keeping the pre-treatment time, the animals were exposed to the pole jump apparatus again. Within 10

trials only the CS, but not the electric foot-shock (UCS) was presented and the time was measured until the rat jumped onto the pole. Each trial was stopped after 20 s. A predefined interval of 30 to 90 s (mean intertrial interval 60 s) was kept between each trial. A reduced CAR in this experiment is considered as antipsychotic activity of the administered drug. Reaction times were integrated over time. Determination of the area under the curve (AUC) values was performed by *Graph Pad Prism*<sup>TM</sup> 3.0. Out of these AUC values for each rat, a dose response curve could be obtained and  $ED_{50}$  values determined.

Immediately after the 10 trials only with CS, five trials were performed where the CS-UCS combination was used again. Time was measured until the rats jumped onto the pole. This step should differentiate between the inhibition of CAR as known for antipsychotic drugs and unspecific motor impairments caused by the administered drugs. Based on reaction times the percentage of conditioned avoidance reaction, avoidance reaction and non-reaction was determined.

#### 2.4. Statistics

All *in vitro* experiments were at least performed twice independently. The outlined results are mean values  $\pm$  SEM (SD for MTT test), calculated by *Microsoft Office Excel*<sup>®</sup> 2007. *In vivo* results were analysed by *SPSS Statistics*<sup>®</sup> 17.0. Locomotor activity measurements were subjected to ANOVA followed by *post hoc* analyses using the *Bonferroni* test. Catalepsy was analysed based on the times the rats remained in the awkward position by ANOVA. CAR times in the pole jump test could not be examined by ANOVA, since the test was stopped at 20 s and there was no normal distribution between the measured times. Hence, nonparametric tests were performed. The significance over all experiments was determined by a *Kruskal-Wallis-H* test and the differences between the different doses to saline solution were examined by *Mann-Whitney-U* tests, followed by *Bonferroni-Holm* correction. Significance between occurrences of non-reactions at different doses was calculated by  $\chi^2$ - and two-sided *Fisher* test. Correlations were calculated according to *Pearson* with two-sided significance. All values were converted into mol units, calculated for the free base. Significance was set at  $p < 0.05$ .

### 3. Results

#### 3.1. *In vitro* tests

Affinities ( $K_i$  values) of the azecines **LE404**, **LE435**, **LE300** and **LE300ME** for dopamine and 5-HT<sub>2A</sub> receptors obtained by radioligand binding experiments are presented in Table 1. For all test compounds  $K_i$  values were in the nanomolar or subnanomolar range. Conduction of a functional

calcium assay revealed, that all compounds act as antagonists at D<sub>1</sub>, D<sub>2</sub> and D<sub>5</sub> receptors.

In general, cytotoxicity of test compounds was examined by using an MTT-test on the human glia cell line U87. Incubation of compounds **LE404**, **LE425** and **LE300** with U87 cells did not reveal any

cytotoxicity up to 250 µM (Table 2). For compound **LE300ME** a weak cytotoxicity was determined (CC<sub>50</sub>=140 µM). Hence, an efficacy/ safety ratio of 1000 was given for all drugs used in the *in vivo* experiments.

**Table 1** Affinities for dopamine D<sub>1</sub> – D<sub>5</sub> and serotonin 5-HT<sub>2A</sub> receptors in radioligand binding experiments

Substance	K <sub>i</sub> [nM] ± SEM					
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>	5-HT <sub>2A</sub>
LE425	28.5±5.2 <sup>a</sup>	13.0±9.0 <sup>a</sup>	75.7±7.3 <sup>b</sup>	43.4±13.3 <sup>a</sup>	54±20.5 <sup>d</sup>	4.42±1.27
LE300	1.9±0.5 <sup>c</sup>	44.7±15.8 <sup>c</sup>	40.4±14.5 <sup>d</sup>	74.9±50 <sup>d</sup>	7.5±0.4 <sup>c</sup>	0.13±0.01
LE404	0.39±0.16 <sup>a</sup>	17.5±1.5 <sup>a</sup>	47.5±16 <sup>b</sup>	7.6±3.7 <sup>a</sup>	1.5±0.5 <sup>a</sup>	0.67±0.04
LE300ME	2.0±0.5	8.2±5.8	7.2±2.8	13.2±2.9	0.5±0.1	0.36
Risperidone	104±43 <sup>e</sup>	4.8±1.3 <sup>e</sup>	13.6±0.47 <sup>e</sup>	12.1±5.0 <sup>e</sup>	563 <sup>e</sup>	0.18±0.02 <sup>e</sup>
Haloperidol	3.4±1.8	0.28±0.26	0.18±0.03	3.5±0.89	4.2±0.3	103±21

<sup>a</sup>data taken from Hoefgen et al. (2006); <sup>b</sup>data taken from Mohr et al. (2006); <sup>c</sup>data taken from Kassack et al. (2002); <sup>d</sup>data taken from Enzensberger et al. (2007); <sup>e</sup>data from PDSP database (National\_Institute\_of\_Mental\_Health, 2010)

**Table 2** Cell viability ± SD after drug administration (100 µM) and calculated CC<sub>50</sub> values (EC<sub>50</sub> equivalent of cytotoxicity) tested on the human glia cell line U87

substance	Viability at 100 µM [%]	CC <sub>50</sub> [µM]
LE425	118.7±4.8	>250µM
LE300	128.0±7.3	>250µM
LE404	120.7±0.5	>250µM
LE300ME	74.0±17.6	139.6±22.7

### 3.2. *In vivo* tests

#### 3.2.1. *Locomotor activity*

Rats were exposed to an open field box 30 and 90 min after drug administration in order to observe changes in behaviour as a result of drug action. Line crossing (horizontal movement), rearing, grooming and defecation were analyzed separately. Grooming and defecation did not change significantly after drug administration. Horizontal and vertical movements revealed parallel dose response curves. Therefore, locomotor activities were measured as sum of both, crossing lines and rearing. Comparing the observations 30 and 90 min after drug administration, **haloperidol** (0.1, 0.2, 0.4 mg/kg), **risperidone** (0.125, 0.4, 0.5 mg/kg), **LE425** (2.0, 5.0, 10.0 mg/kg), **LE300** (1.0, 2.0, 5.0 mg/kg) and **LE300ME** (1.0, 2.0, 5.0 mg/kg) revealed no significant difference between both measurements ( $p>0.05$ ). For **LE404** (0.5, 1.0, 2.0 mg/kg), the effects on locomotor activity seen 30 min after drug administration were significantly reduced for all doses ( $p<0.005$ ) after 90 min. **Haloperidol** ( $F_{3,28}=29.21$ ,  $p<0.001$ ), **risperidone** ( $F_{3,28}=17.96$ ,  $p<0.001$ ), **LE425** ( $F_{3,28}=31.81$ ,  $p<0.001$ ),

**LE300** ( $F_{3,28}=22.21$ ,  $p<0.001$ ), **LE404** ( $F_{3,28}=49.43$ ,  $p<0.001$ ) caused reduced locomotor activity at all investigated doses compared to the control group. **LE300ME** caused a significant decrease in locomotor activity at doses above 1 mg/kg ( $F_{2,21}=40.05$ ,  $p<0.001$ ).  $ED_{50}$  values and 95% confidence intervals are outlined in Table 3. A figure containing the results of the test after 30 min is provided in the supporting information.

#### 3.2.2. *Pole Jump*

Prior to drug testing, all rats were examined on stable conditioned avoidance response (CAR). Only rats which jumped onto the pole within 4 s in four out of five times were used for testing. There were no significant differences in reaction times ( $p>0.05$ ) between the animals used in the experiments. The results for both parts of the experiment (without and with current) are presented graphically for each drug in the supporting information.  $ED_{50}$  values and 95% confidence intervals are outlined in Table 4. For easier understanding, the part without electric foot-shock will in the following be termed as “part A”, and the one with foot-shock will be named “part B”.

#### *Haloperidol*

**Haloperidol** was investigated over five doses in a range from 0.05 to 0.4 mg/kg body weight. The *Kruskal-Wallis-H* test revealed a significant inhibition of CAR ( $p<0.001$ ) of the haloperidol treated animals compared to the saline injected group. Undertaking a *Mann-Whitney-U* test, followed by a *Bonferroni-Holm* correction for each dose, we identified 0.1, 0.15, 0.2

and 0.4 mg/kg as effective doses ( $p < 0.005$ ), whereas 0.05 mg/kg had no effect ( $p > 0.05$ ).

**Table 3**  $ED_{50}$  values and 95% confidence intervals (CI) of locomotor activity (vertical and horizontal movement)

Substance	Locomotor activity $ED_{50}$ [mg/kg]	95% CI
Risperidone	0.30	0.24 – 0.38
Haloperidol	0.10	0.04 – 0.25
LE425	2.09	1.21 – 3.61
LE404	0.41	0.34 – 0.64
LE300	0.74	0.58 – 0.93
LE300ME	1.72	1.22 – 2.41

Part B was conducted in order to investigate an unspecific impairment of the rats to undertake CAR. At 0.05, 0.1 and 0.15 mg/kg there was no increase of escape failures ( $p > 0.05$ ) whereas at doses of 0.2 and 0.4 mg/kg the impairment was significantly higher than in the control group ( $p < 0.01$ ). The dose-dependent decrease in CAR as well as the appearance of non-reactions at doses of 0.2 and 0.4 mg/kg is shown in Figure 3 for all test compounds.

**Table 4**  $ED_{50}$  values and 95% confidence intervals (CI) of CAR in the pole jump experiment

Substance	CAR [mg/kg]	$ED_{50}$ 95% CI
Risperidone	0.61	0.48 – 0.76
Haloperidol	0.20	0.16 – 0.24
LE425	2.51	1.91 – 3.31
LE404	0.32	0.22 – 0.46
LE300	0.94	0.78 – 1.14
LE300ME	0.71	0.60 – 0.85

#### Risperidone

**Risperidone** was examined in four different doses (0.25 – 0.75 mg/kg) and caused a dose dependent significant inhibition of CAR ( $p < 0.001$ ). The investigated doses 0.25, 0.4, 0.5 and 0.75 mg/kg reduced CAR significantly compared to the saline treated animals ( $p < 0.05$ ).

In Part B, CAR-inhibition was significantly increased at 0.4, 0.5, and 0.75, but not at 0.25 mg/kg. No significant impairment on escape behaviour was detected at any investigated dose ( $p > 0.05$ ).

#### LE404

**LE404** significantly increased CAR inhibition over all doses (range 0.1 – 2.0 mg/kg) shown by a *Kruskal-Wallis-H* test ( $p < 0.005$ ). Whereas 0.1 mg/kg revealed no difference compared to the control group (Mann-Whitney-U,  $p > 0.05$ ), a significant enhancement of reaction time compared to the control group was measured for 0.3, 0.5, 1.0 and 2.0 mg/kg ( $p < 0.001$ ).

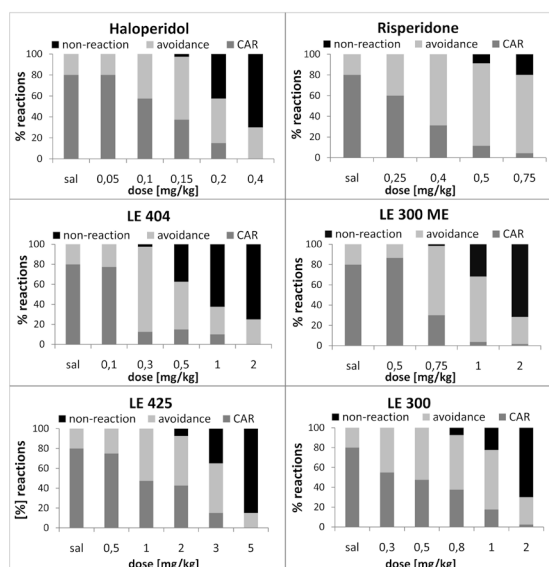
In part B significant CAR-inhibition could be observed for 0.3 – 2.0 mg/kg. An impairment of escape behaviour was only significant for 0.5, 1.0 and 2.0 mg/kg ( $p < 0.05$ ), but not below 0.5 mg/kg ( $p > 0.05$ ).

#### LE300ME

Administration of **LE300ME** enhanced the reaction times at all investigated doses ( $p < 0.01$ ): 0.5 – 2 mg/kg. In part B, CAR-inhibition could not be observed at a dose of 0.5 mg/kg ( $p > 0.05$ ). All other investigated doses, produced a significant reduction of CAR ( $p < 0.001$ ). Increase of non-reaction was observed at doses equal to or higher than 1.0 mg/kg ( $p < 0.001$ ).

#### LE425

In a range from 0.5 to 5.0 mg/kg of **LE425**, a dose dependent increase in reaction time was observed ( $p < 0.001$ ). For 0.5 mg/kg neither an increase in reaction time in part A ( $p > 0.05$ ), nor a significant inhibition of CAR in part B ( $p > 0.05$ ) was observed compared to the control group. For all other doses investigated, starting with 1 mg/kg, enhancement of reaction time and inhibition of CAR were significant ( $p < 0.01$ ). A significant non-reaction occurred only at 3 and 5 mg/kg ( $p < 0.05$ ).



**Figure 3** Effects of test compounds in the pole jump task with current after 4 s: percentage of jumps within 4 s (CAR), after 4 s (avoidance) and no jump within 20 s (non-reaction).

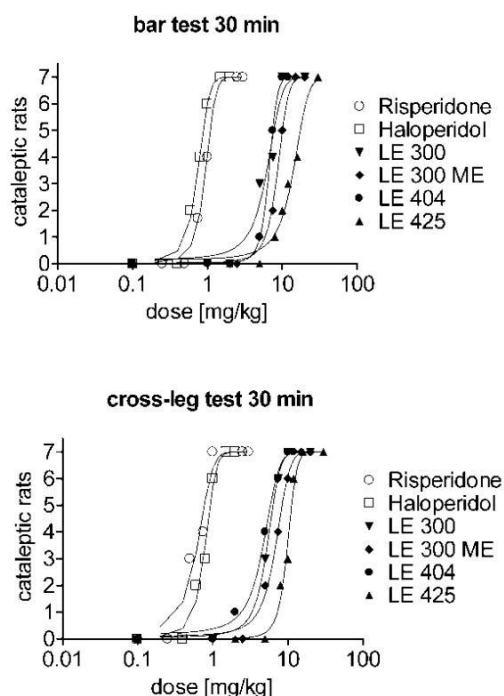
#### LE300

**LE300** was investigated in five different doses ranging from 0.3 to 2.0 mg/kg body weight and showed a dose dependent increase in reaction time over all doses in the *Kruskal-Wallis-H* test ( $p < 0.001$ ). At a dose of 0.3 mg/kg body weight no significant difference compared to the control group was observed ( $p > 0.5$ ), whereas all doses between 0.5 and 2.0 mg/kg caused a significant enhancement of reaction time compared to the animals treated with saline solution ( $p < 0.05$ ). In part B, all doses

influenced CAR significantly ( $p < 0.05$ ). An impairment of the escape behaviour occurred above 2.0 mg/kg ( $p < 0.001$ ).

### 3.2.3. Catalepsy

Results of bar and cross-leg tests 30 min after drug administration are shown in Figure 4. All investigated compounds, including the standards **haloperidol** and **risperidone**, caused catalepsy, stably expressed between 30 and 90 min after drug administration. However, these effects occurred at much higher doses compared to effects on CAR.  $ED_{50}$  values (and 95% confidence intervals) [mg/kg] were as follows: **bar test**: **haloperidol** 0.73 (0.68 – 0.79), **risperidone** 0.90 (0.76 – 1.05), **LE425** 13.92 (12.91 – 15.01), **LE404** 6.54 (5.76 – 7.42), **LE300** 8.84 (7.64 – 10.24), **LE300ME** 8.68 (8.27 – 9.09); **cross-leg test**: **haloperidol** 0.78 (0.70 – 0.87), **risperidone** 0.97 (0.87 – 1.08), **LE425** 12.25 (11.57 – 12.97), **LE404** 6.12 (6.11 – 6.12), **LE300** 7.17 (6.18 – 8.31), **LE300ME** 6.16 (4.22 – 8.97). Obviously, the adverse side-effect catalepsy needed much higher dosages for the new potential LE-type-neuroleptics than for the established reference neuroleptics **haloperidol** and **risperidone**.

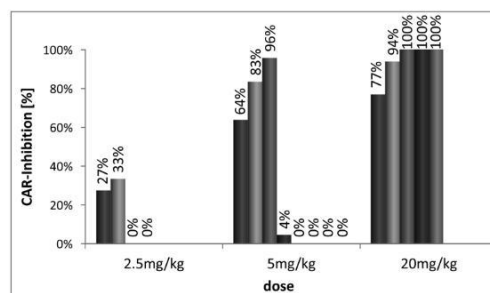


**Figure 4** Dose-dependence of bar test and cross-leg test 30 min after drug administration. Rats ( $n = 7$  per dose) which remained  $\geq 90$  s in an awkward position were counted as cataleptic.

### 3.2.4. Oral bioavailability

In order to examine the oral bioavailability of the investigated azecines, 2.5 mg/kg of **LE404** and **LE300ME** were administered per os to four rats each and the treated animals were exposed to the pole jump test 45 min (**LE404**) or 60 min (**LE300ME**) after

drug administration. At this dose **LE300ME** revealed a  $74.5 \pm 19.2\%$  CAR-inhibition. All rats were significantly influenced by the peroral administered drug ( $p = 0.004$ , data not shown). Compound **LE404** could only induce a CAR-inhibition of  $30.1 \pm 2.1\%$  in two out of four rats at this dose, not enhanced by variation of the pre-treatment time (30 to 90 min). Further dose-increase of **LE404** to 5 mg/kg body weight ( $n = 8$  rats) led to similar results (Figure 5). Only three out of 8 rats were affected at this higher dose, with an enhanced CAR-inhibition rate of  $80.9 \pm 8.8\%$ . At a dose of 20 mg/kg ( $n = 5$ ), all investigated animals showed CAR-inhibition of  $94.1 \pm 4.5\%$ . Three out of five rats displayed catalepsy at this dose (bar test).



**Figure 5** Oral bioavailability of LE404 at different doses measured in the pole jump test as percentage of CAR-inhibition (each bar represents one rat).

## 4. Discussion

Dopamine receptor antagonists, in first line those with prevalence for the  $D_2$  subtype, are known as potent drugs against the positive symptoms of schizophrenia, but often cause therapy limiting side-effects. Parkinson like symptoms, often induced by these drugs, are considered to be reduced by serotonin 2A receptor antagonism. The azecines described in this study were structurally designed to combine both, potency against positive symptoms by dopamine antagonism and reduced side-effects by serotonin antagonism in one drug. This class of new substances has brought up some nanomolar antagonists at human dopamine ( $D_1 - D_5$ ) receptors with prevalence to the  $D_1$  family. In the current work, we investigated *in vitro* some of these potential antipsychotics also at the human 5-HT<sub>2A</sub> receptor, measured their cytotoxic effects and conclusively their efficacy *in vivo*.

Based on the results of *in vitro* testing, we selected the four azecine derivatives **LE300**, **LE300ME**, **LE404** and **LE425** for *in vivo* investigations. Besides the high affinities for dopamine and 5-HT<sub>2A</sub> receptors, a low general cytotoxicity of the compounds was a basic requirement for the selection. Fortunately, all investigated compounds showed nanomolar affinities to  $D_1 - D_5$  and 5-HT<sub>2A</sub> receptors and  $CC_{50}$  values on human glia cells in cytotoxicity assays above 100  $\mu M$ . Previous studies have shown that all clinical relevant neuroleptics inhibit selective conditioned avoidance



response (CAR) at doses that do not influence the natural escape behaviour (Wadenberg and Hicks, 1999). The suppression of CAR in rats has a high predictive validity for antipsychotic potency (Kuribara and Tadokoro, 1981). Therefore we studied the neuroleptic like potency of our four test compounds by a one-way active avoidance task in the pole jump model.

In our experiments, **haloperidol** and **LE404** were equally potent in CAR inhibition and **LE300ME** was as potent to reduce CAR as **risperidone**. **LE300** and **LE425** showed the same effects, but only at higher doses. All tested azecines inhibited CAR potently at doses, at which the escape behaviour was not restrained. From these results we conclude that the tested azecines have a high likelihood of neuroleptic potency to treat positive symptoms of schizophrenia.

It might be argued, that the effect of CAR is only a consequence of reduced locomotor activity. So we measured locomotor activity and indeed found it to be decreased by all test compounds. However, the calculated  $ED_{50}$  values of the locomotor activity inhibition do only correlate moderately with the suppression of CAR ( $r^2=0.65$ ,  $p=0.05$ ). Some mild correlation is reasonable, due to the fact that both, locomotor activity and antipsychotic efficacy are at least partially caused by the same mechanism – dopamine antagonism. Nevertheless, there are published examples of antipsychotics, where CAR inhibition occurs at doses, at which the locomotor activity is not influenced (Becker and Grecksch, 2008). In the present study, **LE300ME** showed significantly less reduction in locomotor activity than effects on CAR.

Next to the improvement of symptoms, a high therapeutic range regarding side-effects is a main demand on new neuroleptics. Extra-pyramidal movement disorders are some of the most common adverse side-effects of antipsychotics and are often therapy limiting. Therefore we examined the induction of catalepsy, a reliable animal model for these Parkinson like symptoms, for all test substances. **Haloperidol** showed catalepsy at very low doses ( $<1$  mg/kg), while the  $ED_{50}$  values of the investigated azecines ranged between 6 and 16 mg/kg body weight. **LE404** even had a 5-fold broader therapeutic range regarding catalepsy than the investigated standard compounds (compare Table 5). **LE300** and **LE300ME** revealed mild advantages compared to **haloperidol** and **risperidone** in the catalepsy-potency-ratio. It is remarkable, that the azecine derivatives but not **risperidone**, an often prescribed atypical antipsychotic, show advantages over **haloperidol** in this model. Nevertheless, **risperidone** itself is mainly used because of its equally effect on negative and positive symptoms. The occurrence of extra-pyramidal side-effects (EPS) in patients at

higher doses of **risperidone** is well known. In several studies clinical titrated doses of **haloperidol** and **risperidone** showed no significant difference in the occurrence of EPS (Ceskova and Svestka, 1993; Chouinard et al., 1993). The standard compounds **haloperidol** and **risperidone** are accepted and commonly used drugs in the treatment of schizophrenia. Regarding the receptor binding profile, the typical antipsychotic **haloperidol** displayed high binding affinities to all dopamine receptors, but prevalence for the  $D_2$  and  $D_3$  receptors. It showed only very low affinity for the  $5-HT_{2A}$  receptor. **Risperidone** as an example for atypical antipsychotics showed selectivity to the  $D_2$ ,  $D_3$ ,  $D_4$  receptors, but also high affinities for the  $5-HT_{2A}$  receptor in a subnanomolar range. **LE300**, **LE300ME**, **LE404** and **LE425** revealed affinities for the  $5-HT_{2A}$  receptor comparable with those of **risperidone**, but much higher affinities for  $D_1$  and  $D_5$ . The different subreceptor affinities and selectivities of the test compounds should enable us to investigate the influence of the different dopamine receptor subtypes and the  $5-HT_{2A}$  receptor on potency against positive symptoms and induction of catalepsy. Using the results from open field tests (locomotor activity), pole jump tests (CAR-inhibition, antipsychotic potency) and bar tests (catalepsy), we tried to correlate the data of the *in vitro* receptor binding studies with the ones obtained in animal models *in vivo*. A distinct correlation between the affinities for a single receptor subtype to the  $ED_{50}$  values of the different animal tests could not be found ( $p>0.05$ ). Best results, but with no significance were observed for  $D_2$ /locomotor activity ( $r^2=0.71$ ,  $p=0.22$ ),  $D_4$ /CAR-inhibition ( $r^2=0.63$ ,  $p=0.59$ ) and  $D_2$ /catalepsy ( $r^2=0.64$ ,  $p=0.16$ ). Surprisingly there was a strong and significant correlation between the CAT(C-L)/CAR ratio and  $D_1$  ( $r^2=0.95$ ,  $p=0.03$ ) or  $D_5$  receptors ( $r^2=0.96$ ,  $p=0.03$ ), which could imply an advantage of high affinities to the  $D_1$  receptor family, displayed by almost all hexahydrodibenz- and hexahydrobenzindoloazecines. Some formerly postulated correlation between  $5-HT_{2A}$  receptor affinities and catalepsy (Balsara et al., 1979) or CAT/CAR-ratio, respectively, could not be found for the tested compounds ( $p>0.5$ ). This missing correlation as well as the results for **risperidone** indicate that the  $5-HT_{2A}$  receptor affinity might not be responsible for less extra-pyramidal side-effects as also discussed by Kapur et al. (Kapur, 1996). Further investigations on azecines should therefore concentrate on the influence of the additional  $5-HT_{2A}$  affinities on negative symptoms.

Dopamine hypofunction in the prefrontal cortex, postulated as crucial for negative symptoms, might be reduced due to the possible dopamine release into this brain region by  $5-HT_{2A}$  receptor blockade (Davis et al., 1991).



**Table 5**  $ED_{50}$  values of the *in vivo* tests and determination of the CAT/CAR-inhibition ratio

Substance	Locomotor activity $ED_{50}$ [mg/kg]	CAT (bar) [mg/kg]	$ED_{50}$	CAT (CLP) $ED_{50}$ [mg/kg]	CAR $ED_{50}$ [mg/kg]	CAT/CAR
Risperidone	0.3	1.2		1.1	0.61	1.8 – 2.0
Haloperidol	0.1	0.7		0.8	0.20	3.5 – 4.0
LE425	2.1	13.9		7.2	2.51	2.8 – 5.5
LE404	0.4	6.5		4.3	0.32	13.4 – 20.3
LE300	0.7	8.8		7.2	0.94	7.7 – 9.4
LE300ME	1.7	8.3		6.2	0.71	8.7 – 11.7

CAT (bar) –catalepsy (bar test); CAT (CLP) – catalepsy (cross-leg-position test); CAR – conditioned avoidance reaction

Beside the therapeutic effect itself and few side-effects, oral bioavailability is a basic requirement for antipsychotic drugs. We investigated one example of both scaffolds (dibenz- and benzindolo[*d,g*]azecines) by oral administration in the pole jump test. The benzindoloazecine **LE300ME** showed constantly good results at the investigated dose of 2.5 mg/kg (79.6% CAR-inhibition). All rats were influenced at this dose ( $n=4$ ). Results for the dibenzazecine **LE404** were more difficult to interpret. Some rats showed distinct inhibition of CAR at 2.5 and catalepsy at 20 mg/kg, whereas other rats showed CAR-inhibition only at 20 mg/kg with no effect on escape behaviour or catalepsy. Hence, we assume that the oral bioavailability of **LE404** may be inter-individually different. Though we cannot explain this observation so far, an influence of the phenolic hydroxy function of this substance on oral bioavailability is conceivable.

## 5. Conclusion

Aromatic annelated [*d,g*]azecines represent a novel and advantageous type of potential neuroleptics. They were investigated with respect to serotonin receptor affinity, cytotoxicity, *in vivo* antipsychotic potency (pole jump test, CAR), extra-pyramidal side-effects (catalepsy) and oral bioavailability. Four examples of this substance class, each with different selectivity profile to dopamine receptors, were selected for the experiments. All of them showed to be potent in an animal model for antipsychotic activity. **LE404** and **LE300ME** revealed activities against positive symptoms comparable to the standard compounds **haloperidol** and **risperidone**. **LE300** and **LE425** showed the same effects on CAR albeit at higher doses. By these observations, we could prove for the first time the ability of the investigated azecines to pass the blood-brain-barrier. The tested azecines caused catalepsy only at significantly higher doses than **haloperidol** and **risperidone**. Hence, three of four substances revealed a beneficial therapeutic range compared to the standard compounds regarding extra-pyramidal side-effects. **LE404**, the best compound in this study, was about 5 times better than **haloperidol** or **risperidone**. Analysing the CAT/CAR-ratio for all

tested compounds, their possible clinical value based on the animal experiments can be ranged as follows: **LE404** > **LE300ME** ≥ **LE300** > **LE425** ≥ **haloperidol** ≥ **risperidone**. Nevertheless, this should be only seen as tendency and is maybe not transferable to the effects in humans. In addition we investigated the effects on CAR of the two most promising compounds after oral administration. **LE404** displayed a heterogeneous result when administered orally, most probably due to the phenolic function. Only some of the animals behaved as expected, while others needed a much higher dose for the same outcome. However, compound **LE300ME** showed constant CAR-inhibition already at low doses after oral administration. We conclude that the investigated azecines represent a class of substances with interesting characteristics of potential neuroleptics. Beside the high dopamine and serotonin receptor affinities and low cytotoxicity, these compounds showed high potency against positive symptoms *in vivo*, less extra-pyramidal side-effects than **risperidone** and probably acceptable oral bioavailability.

## Acknowledgement

The authors would like to thank Prof. Gisela Grecksch for introducing us to the pole jump experiment as well as Anne Berthold and Anne-Katja Imhof for the expert assistance in drug administration.

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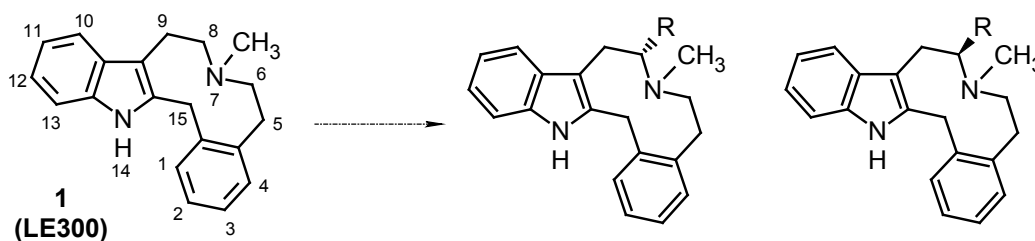
## Paper 4

### Synthesis and Dopamine Receptor Affinities of Racemic and Enantiopure Indolo[3,2-*f*][3]benzazecine Derivatives

Dina Robaa, Christoph Enzensperger, Mohamed M. Hefnawy, Hussein I. El-Subbagh, Shams EIDin AbulAzm, and Jochen Lehmann

*J. Med. Chem.*; manuscript in preparation

**Abstract:** LE300 derivatives substituted at position 8 with three different residues, namely methyl, hydroxymethyl and carboxylic acid, were prepared. The 8-methyl and 8-hydroxymethyl derivatives were obtained as the separated *R*- and *S*- enantiomer as well as the racemic mixture, while the amino acid derivative could only be obtained in a racemized form. The separate enantiomers showed significantly different affinities; the 8*S*-methyl and 8*R*-hydroxymethyl derivatives, where the substituent points behind the plane, were markedly more active than their enantiomeric counterparts. The racemized derivative substituted with a carboxylic acid group, though it could not be in an enantiopure, was highly interesting since it displayed a pronounced selectivity for the D<sub>5</sub>-receptors.



**Contribution:** Synthesis and characterization of all target and intermediary compounds, separation of the racemate by preparative chiral HPLC, determination of  $K_i$  values of some derivatives by radioligand binding assay, development of SAR and preparation of the manuscript.



# Synthesis and Dopamine Receptor Affinities of Racemic and Enantiopure Indolo[3,2-*f*][3]benzazecine Derivatives

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Racemic and enantiopure 8-substituted derivatives of the lead dopamine receptor antagonist LE 300 (**1**) were prepared and their affinities for dopamine receptors (D<sub>1</sub>-D<sub>5</sub>) were tested. The separate enantiomers showed significantly different affinities; 8*S*-methyl and 8*R*-hydroxymethyl derivatives where the substituents point behind the plane, were markedly more active than their enantiomeric counterparts. The racemized derivative substituted with a carboxylic acid group was selective for the D<sub>5</sub>-receptor.

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## INTRODUCTION

Dopamine is known as a major neurotransmitter in the CNS, where it controls numerous physiological functions, including locomotion, behaviour, emotion, cognition, learning and motivation. Dysfunctions of the dopaminergic system have been linked with several neurological and psychiatric disorders mainly Parkinson's disease<sup>1,2</sup> and schizophrenia.<sup>3,4</sup> Dysregulations of the dopamine system have also been associated with depression<sup>5</sup>, attention-deficit hyperactivity disorder<sup>6</sup> and alcohol dependence.<sup>7</sup>

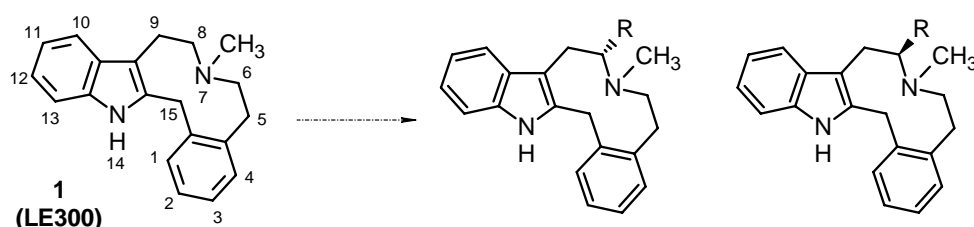
Partially hydrogenated indolo[3,2-*f*][3]benzazecines,<sup>8,9</sup> represent a structurally novel class of dopamine receptor antagonists, distinguished by nanomolar affinities as well as their preference for the receptors of the D<sub>1</sub> family. Their structure is typically made up of a tetracyclic ring system; with two aromatic moieties surrounding a central azecine ring. Since the discovery of 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecines (**1**, LE300),<sup>9</sup> the lead benzindoloazecine derivative, extensive research has been devoted to the search for derivatives showing either improved affinities and/or subtype selectivity. Considerable work has been accomplished in developing SAR of this class of compounds.<sup>8,10,11</sup> This revealed that the backbone scaffold is optimally made up of a two aromatic ring system (indole and benzene, or two benzene rings) surrounding a central azecine moiety. Both aromatic rings should be separated by a methylene group. Further studies showed that small substituents at some positions of the benzindoloazecine scaffold, e.g. at the indole nitrogen, are well tolerated or may even result in increased affinities.<sup>12</sup>

Aim of this work was to prepare the first indolo[3,2-*f*][3]benzazecine derivatives (benzindoloazecines) substituted at a C-atom of the azecine ring besides the methyl group at the azecine nitrogen. We selected position 8 in order to test the effect of a substituent placed nearby the azecine nitrogen on the affinities and

selectivities of the compounds. Since this type of substitution results in the formation of a chiral centre, we wanted to obtain not only the racemic but also the enantiopure derivatives in order to additionally assess the effect of the stereochemistry.

Enantiomeric pairs commonly show different activities, pharmacokinetics or even toxicity. Diverse dopamine receptor ligands are chiral in nature, their chirality originating mostly at the central ring, mainly affecting the spatial conformation of the scaffold as a whole. (*cf.* apomorphines,<sup>13</sup> phenylbenzazepines<sup>14</sup> and dihydroexidine<sup>15</sup>). Our notion was that an additional substituent at the 8-position of the benzindoloazecine molecule might similarly affect the conformation of the whole scaffold, depending on the configuration of the substituent (*R*- or *S*-).

**Chart 1.** Substitution of the lead benzindoloazecine derivative **1** at position 8.



Our primary targets were racemic and enantiopure benzindoloazecine derivatives substituted at position 8 with a small residue such as a methyl group. Based on the obtained results, the nature of the substituent was to be modified by introduction of a functional group such as a carboxyl or hydroxyl group.

## CHEMISTRY

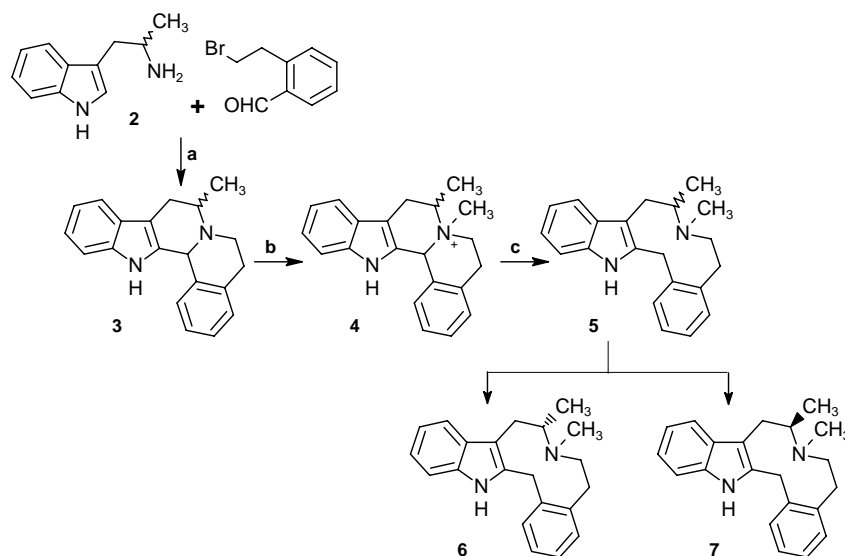
To obtain the *R*- and *S*-enantiomers of the target 8-methylated benzinodoloazecines (**6** and **7**) racemic  $\beta$ -methyltryptamine (**2**) was first converted to the quinolizine derivative **4** by reaction with 2-(2-bromoethyl)benzaldehyde following a previously established procedure for the synthesis of analogous quinolizines.<sup>16</sup> Subsequent quaternization with methyl iodide and reduction of the obtained quaternary salts under Birch conditions yielded the target azecine as a racemate **5**.

Resolution of the racemate to attain the enantiomers **6** and **7** was achieved by means of preparative chiral HPLC on a cellulose-based chiral stationary phase. Several attempts to crystallize the obtained enantiopure derivatives to obtain an X-ray structure and in turn assign the respective configuration were not successful.

The quinolizine derivatives **10** and **11**, obtained from the enantiomerically pure methyl esters of D- and L-tryptophan (**8** and **9**), served as starting materials for the preparation of further 8-substituted LE300 derivatives. Conversion of the quinolizines **10** and **11** to the corresponding azecines however failed at the last step, namely at the reduction of the quaternary salts **12** and **13** under Birch conditions.

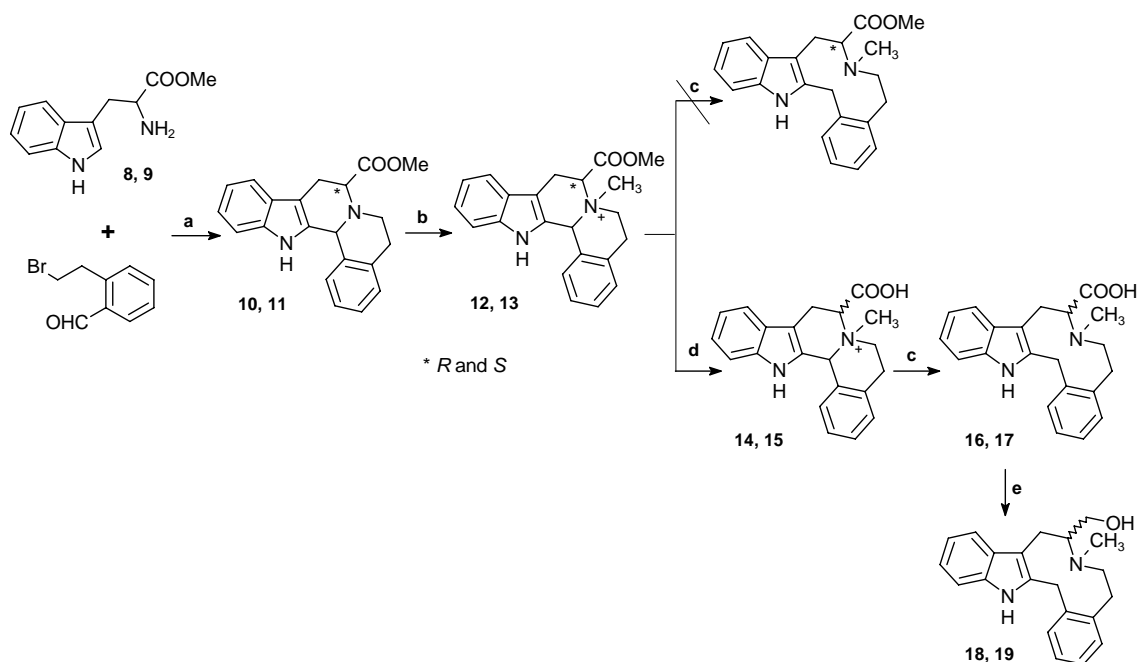


**Scheme 1<sup>a</sup>.** Synthesis of the 8-methyl benzinodoloazecine derivative **5** and its enantiomeric separation.



<sup>a</sup> Reagents and conditions: (a) TFA, dioxane, reflux, 6h; (b) MeI, acetone, 24 h; (c) Na<sup>0</sup>, liq. NH<sub>3</sub>, -40°C, 10 min.

**Scheme 2a.** Synthetic route A for the preparation of benzinodoloazecines derived from D- and L-tryptophan.



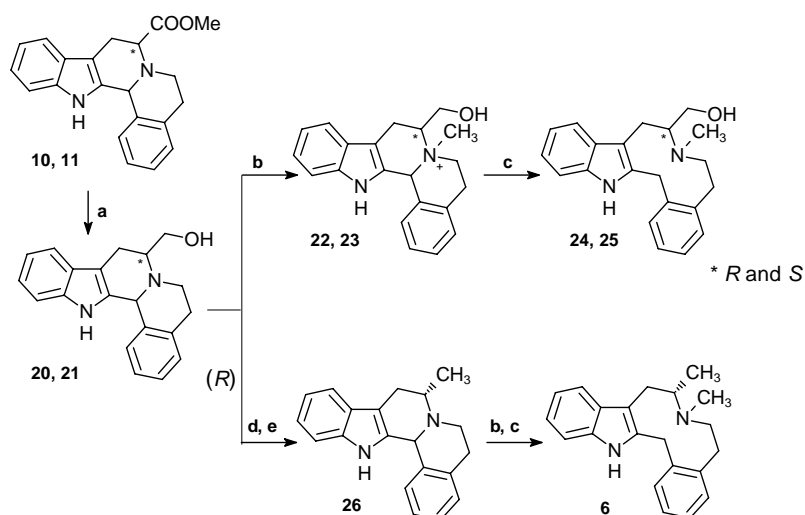
<sup>a</sup> Reagents and conditions: (a) TFA, dioxane, reflux, 3h; (b) MeI, acetone, 35 °C, 24 h; (c) Na<sup>0</sup>, liq. NH<sub>3</sub>, -40°C, 10 min; (d) 1N NaOH, MeOH, 0°C, 2 h; (e) LiAlH<sub>4</sub>, dry THF, reflux, 5 h.

As an alternative route, the ester function of the quaternary salts **12** and **13** were first hydrolyzed in 1N sodium hydroxide at 0 °C, and the resulting products **14** and **15** were reduced under Birch conditions yielding the amino acid azecine derivatives **16** and **17**. These were in turn reduced using lithium aluminium hydride to

give the hydroxymethyl derivatives **18** and **19**. (**Scheme 2**) However, investigations of the enantiomeric purity of the obtained derivatives **18** and **19** by means of chiral HPLC as well as measurement of their optical rotation revealed that these derivatives were obtained in an almost completely racemized form. It can be presumed that this racemisation occurred due to the basic conditions used for the hydrolysis of the ester function of the quaternary salts **12** and **13** to the corresponding carboxylic acid derivatives **14** and **15**, despite the mild conditions applied namely at 0 °C for 2 h.

In a further trial to obtain enantiopure derivatives, the hydrolysis step was circumvented, and the ester function of the quinolizines **10** and **11** was first reduced using lithium aluminium hydride. The resulting hydroxymethylquinolizines **20** and **21** were subsequently quaternized, and the quaternary salts **22** and **23** were finally reduced with sodium in liquid ammonia to give the target azecines **24** and **25**. (**Scheme 3**)

**Scheme 3<sup>a</sup>**. Synthetic route B: 8-Hydroxymethyl-quinolizine as starting material for enantiopure 8-hydroxymethyl and 8-methyl benzindoloazecines



<sup>a</sup> Reagents and conditions: (a) LiAlH<sub>4</sub>, dry THF, rt, 90 min; (b) MeI, acetone, 24 h; (c) Na<sup>o</sup>, liq. NH<sub>3</sub>, -40°C, 10 min; (d) MsCl, TEA, DCM, 12 h, rt; (e) LiAlH<sub>4</sub>, dry THF, reflux, 2h.

The enantiopurity of the obtained azecines was ascertained by measuring their specific rotation ( $[\alpha]_{495}^{rt} = -158.4^\circ$  and  $161^\circ$ , respectively) and by chiral HPLC. These findings substantiate our earlier speculations that in the previously adopted synthetic procedure racemisation took place at a step prior to the synthesis of the hydroxymethyl derivatives **18** and **19**, and that it most probably occurred during hydrolysis. It could also be concluded that the amino acid derivatives **16** and **17** are not enantiomerically pure and that they are racemized to the same extent as the hydroxymethyl derivatives **18** and **19**. In order to identify the configuration of the aforementioned separated *S*- and *R*-enantiomers of 8-methyl benzindoloazecine (**6** and **7**; **scheme 1**), we adapted an alternative route for the synthesis of the (*8S*)-enantiomer (**6**) starting from an enantiomerically pure precursory substance, namely the *8R*-hydroxymethyl quinolizine **20**. This was first converted to the corresponding *R*-mesylate, which was directly reduced with lithium aluminium hydride to give the (*8S*)-methyl

quinolizine **26**. Reduction with sodium in liquid ammonia finally yielded the corresponding (8*S*)-azecine derivative **6**. (**Scheme 3**) Comparison of the respective specific rotation of the herein obtained azecine with the previously obtained ones (**scheme 1**; **6** and **7**) enabled us to assign the respective configuration.

## PHARMACOLOGY

All the target enantiopure compounds (**6**, **7**, **24** and **25**) together with their racemic forms (**5** and **18**) as well as the racemized amino acid derivative **16** and **17** were screened for their affinities for the human cloned dopamine receptor subtypes D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, D<sub>4.4</sub> and D<sub>5</sub>. These receptors were stably expressed in HEK293 or CHO cells; [<sup>3</sup>H]SCH 23390 and [<sup>3</sup>H]spiperone were used as radioligands at the D<sub>1</sub>-like and D<sub>2</sub>-like receptors, respectively. *K<sub>i</sub>* values are given in nanomolar units (**Table 1**). A detailed protocol has been elsewhere described.<sup>16</sup> Additionally, the compounds were tested in an intracellular Ca<sup>2+</sup> assay, in order to determine their functionality at the D<sub>1</sub> and D<sub>2</sub> receptors. HEK293 cells stably expressing the respective D-receptor were loaded with a fluorescent dye (Oregon green), and after preincubation with rising concentrations of the test compound, an agonist (SKF 38393 for D<sub>1</sub> and quinpirole for D<sub>2</sub>) was added and the Ca<sup>2+</sup>-induced fluorescence was measured with NOVOSTAR<sup>®</sup> microplate reader. The ability of the test compound to suppress the agonist-induced Ca<sup>2+</sup> influx is an indication of antagonistic or inverse agonistic properties at the receptor.

## RESULTS and DISCUSSION

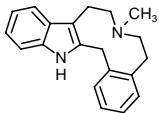
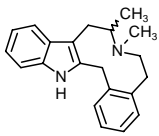
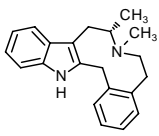
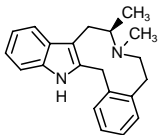
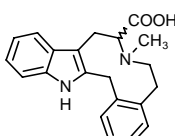
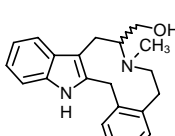
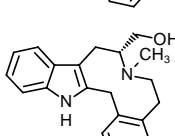
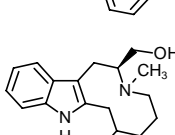
Benzindoloazecine derivatives substituted at position 8 with three different residues, namely methyl, hydroxymethyl and carboxylic acid, were prepared. The 8-methyl and 8-hydroxymethyl derivatives were obtained as the separated *R*- and *S*- enantiomer (**6**, **7** and **24**, **25**, respectively) as well as the racemic (**5**) or racemized (**18**, **19**) mixture, while the amino acid derivative could only be obtained in a racemized form (**16+17**).

All racemates showed decreased affinities for the dopamine receptors compared with the lead benzindoloazecine **1**; the most pronounced decrease was observed with the amino acid derivative **16**. This was however interesting, since compound **16** displayed practically no affinities for all dopamine receptors (*K<sub>i</sub>* > 10000 nM), except for the D<sub>5</sub> receptor, where it showed submicromolar one (ca. 657 nM). This apparent selectivity for D<sub>5</sub> against D<sub>1</sub> is seldom encountered, which renders the compound highly attractive as a potential D<sub>5</sub>-selective pharmacological tool. To attest this D<sub>5</sub>/D<sub>1</sub> selectivity a competitive binding experiment at the D<sub>1</sub> receptor (determination of *K<sub>i</sub>* value) was carried out, not to only rely on the results of the screening. Separation of the enantiomers of compound **16** could not be accomplished, but it would be of great value, as the separate enantiomers could show higher affinities or more pronounced selectivity.

While the racemic (8*RS*)-methyl benzindoloazecine **5** showed significantly decreased affinities (radioligand binding experiment), the separated *R*- and *S*- enantiomers displayed a high discrepancy in their affinities. The *S*-enantiomer **6** (bond pointing below the plane) was almost as active as the lead benzindoloazecine **1**. The corresponding *R*-isomer **7** exhibited at least 100-fold reduction in affinities for all dopamine receptors, showing

micromolar affinities for D<sub>1</sub>-like receptors (D<sub>1</sub> and D<sub>5</sub>) and was practically devoid of activity for D<sub>2</sub>-like receptors (D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>).

**Table 1:** Affinities ( $K_i$ , nM) for human D<sub>1</sub>-D<sub>5</sub> receptors, determined by radioligand binding experiments

Compounds	$K_i$ , (nM)				
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
<b>1*</b> 	1.9 ± 0.9	44.5 ± 15.8	40.3 ± 4.4	108 ± 39	7.5 ± 0.3
<b>5</b> 	32.4 ± 13.3 <sup>b</sup>	526 ± 69 <sup>b</sup>	385 ± 3.5 <sup>b</sup>		16.9 ± 6.5 <sup>b</sup>
<b>6</b> 	6.1 ± 2.2 <sup>a</sup>	85.3 ± 15.2 <sup>b</sup>	304 ± 1 <sup>b</sup>	64.1 ± 19.3 <sup>b</sup>	4.4 ± 2.5 <sup>b</sup>
<b>7</b> 	640 ± 119 <sup>b</sup>	>10000	>10000	>10000	389 ± 0.8 <sup>b</sup>
<b>16</b> 	>10000	>10000	>10000	>10000	657 ± 163 <sup>a</sup>
<b>18</b> 	44.8 ± 29.6 <sup>b</sup>	200.7 ± 89.4 <sup>a</sup>	>10000	512 ± 274 <sup>b</sup>	93.2
<b>24</b> 	10.3 ± 1.4 <sup>b</sup>	268 ± 56 <sup>a</sup>	681 ± 68 <sup>a</sup>	408 ± 31 <sup>b</sup>	11.4 ± 2.3 <sup>b</sup>
<b>25</b> 	114 ± 9 <sup>b</sup>	2923 ± 442 <sup>a</sup>	4676 ± 1593 <sup>b</sup>	4861 ± 803 <sup>b</sup>	91.4 ± 8.5 <sup>b</sup>

\*Values taken from ref. 10. CHO cell lines were used for D<sub>4.4</sub>; HEK cell lines for D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, and D<sub>5</sub>. <sup>a</sup> $K_i$  values are the means of three experiments; performed in triplicate ± SEM. <sup>b</sup> $K_i$  values are the means of two; experiments; performed in triplicate ± SEM.

A similar but less pronounced difference was observed between the *R*- and *S*- enantiomers of 8-hydroxymethyl benzindoloazecine, the *R*-enantiomer **24** (bond also pointing below the plane) was more active than the *S*-enantiomer **25**, however only a tenfold difference in affinities was found. While the *R*- enantiomer of 8-

hydroxymethyl benzindoloazecine was relatively less active than its 8-methylated counterpart (the *S*-enantiomer; in both compounds the bond points below the plane), the (8*S*)-hydroxymethyl derivative was more active than the (8*R*)-methylated one.

## EXPERIMENTAL SECTION

**General methods:** Melting points are uncorrected and were measured in open capillary tubes using a Gallenkamp melting point apparatus. <sup>1</sup>H and <sup>13</sup>C NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS using a Hewlett-Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Purities of the compounds were determined by elemental analysis, performed on a Hereaus Vario EL apparatus, or by HPLC. All values for C, H, and N were found to be within (±0.4). All compounds showed > 95% purity.

**General Procedure for the preparation of the quinolizines 3, 10 and 11 starting from the respective amines:** A solution of the respective aryethylamine (N-methyltryptamine, methyl D- or L-tryptophane) (1 mmol), 2-(2-bromoethyl)benzaldehyde (1.2 mmol) and trifluoroacetic acid (1 mmol) in dioxane was refluxed under nitrogen for three to six hours (with N-methyltryptamine). The solvent was then evaporated under reduced pressure, the remaining residue basified with 2N NaOH and the resulting quinolizine base was extracted with dichloromethane.

**(8*RS*,14*bRS*)-8-Methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinoline (3).** The obtained product was crystallized from isopropanol/hexane giving pale yellow needle-shaped crystals; yield 69.5%; mp: 75-77 °C. <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): 1.41-1.48 (2d, *J*=7, 3H, CH<sub>3</sub>), 2.50-3.25 (m, 6H, aliphatic), 3.46-3.67 (m, 1H, H-8), 5.24 and 5.41 (2s, 1H, H-14b), 7.07-7.51 (m, 8H, aromatic), 7.66 and 7.72 (2s, 1H, indole-*NH*). GC-MS: two peaks of the same molecular weight were detected, which correspond to the two diastereomers formed during the reaction: m/z: 288 (53%), 273 (23%), 245 (63%), 230 (99%), 217 (74%), 202 (14%), 156 (22%), 143 (16%), 130 (100%), 115 (96%), 103 (40%), 89 (29%), 77 (83%), 63 (33%). And m/z: 288 (%), 273 (23%), 245 (63%), 230 (99%), 217 (74%), 202 (14%), 156 (22%), 143 (16%), 130 (100%), 115 (96%), 103 (40%), 89 (29%), 77 (83%), 63 (33%). And m/z: 288 (53%), 273 (23%), 245 (63%), 230 (99%), 217 (74%), 202 (14%), 156 (22%), 143 (16%), 130 (100%), 115 (96%), 103 (40%), 89 (29%), 77 (83%), 63 (33%). And m/z: 288 (44%), 273 (19%), 245 (51%), 230 (83%), 217 (70%), 202 (19%), 156 (20%), 143 (11%), 130 (100%), 115 (92%), 103 (40%), 91 (34%), 77 (86%), 63 (31%).

**Methyl (8*R*)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinoline-8-carboxylate (10).** Purified by column chromatography, dichloromethane/methanol (100:1). Yield 55.2%; m.p. 84-87 °C (analytical data see SI).

**Methyl (8*S*)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinoline-8-carboxylate (11).** Purified by column chromatography, dichloromethane/methanol (100:1). Yield 59.1%; m.p. 84-86 °C (analytical data see SI).

**General Procedure for the Preparation of the Quaternary Salts 4, 12, 13, 22 and 23.** To a stirred solution of the respective quinolizine in acetone was added a 10-fold molar excess of methyl iodide. Stirring was continued for 24 h, the precipitated solid was filtered off and the filtrate treated with excess diethyl ether to precipitate the rest of the quaternary salt. Both precipitates were collected, washed with diethylether and dried.

**(8*RS*)-7,8-Dimethyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinolin-7-ium iodide (4).** Yellow solid. Yield 86.4%; m.p. 274-275 °C. <sup>1</sup>H NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 1.53-1.57 and 1.63-1.65 (2d, *J*= 6, 3H, CH<sub>3</sub>), 2.85-3.27 (m, 4H, aliphatic), 3.10 and 3.16 (2s, 3H, N-CH<sub>3</sub>), 3.50-3.69 (m, 1H, aliphatic), 4.03 and 4.26-4.20 (2m, 2H, aliphatic), 6.12 and 6.22 (2s, 1H, H-14b), 7.00-7.21 (m, 2H, aromatic), 7.36-7.65 (m, 6H, aromatic), 10.20 and 11.48 (2s, 1H, indole-*NH*).

**(8*R*)-(Methoxycarbonyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinolin-7-ium iodide (12).** Yellow solid. Yield 88%; m.p. 217-218 °C (analytical data see SI).

**(8*S*)-(Methoxycarbonyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinolin-7-ium iodide (13).** Yellow solid. Yield 85%; m.p. 215-216 °C (analytical data see SI).

**(8*R*)-8-(Hydroxymethyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]iso-quinolin-7-ium iodide (22).** Yellow solid. Yield 99.1%; m.p. >300 °C Used without further purification for subsequent syntheses (analytical data see SI).

**(8*S*)-8-(Hydroxymethyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]iso-quinolin-7-ium iodide (23).** Yellow solid. Yield 95.0 %; m.p. > 300 °C. Used without further purification for subsequent syntheses.

**General Procedure for the Ring-Opening.** Ammonia was condensed in a three-necked 100 mL flask, which was equipped with a balloon and a stopper and cooled in a liquid nitrogen bath. After filling 3/4 of the flask's volume, the cooling bath was removed and ammonia was allowed to liquefy. The respective quaternary salts were then added to the stirred liquid ammonia. This was followed by gradual addition of small pieces of sodium metal until the blue colour remained for 10 min. A few drops of saturated ammonium chloride solution were added to terminate the reaction, and the mixture was stirred under nitrogen until the liquid ammonia completely evaporated. To the residue was added 10 mL water, and the mixture was then extracted with 30 mL diethyl ether. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was removed under reduced pressure.

**(8*RS*)-7,8-Dimethyl-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (5) and its separated *R*- and *S*-enantiomers 6 and 7.** Creamy white solid. Yield 89.7%; m.p. 82-84 °C. <sup>1</sup>H NMR: 400 MHz (CDCl<sub>3</sub>): δ 1.09-1.11 (d, *J*=7, 3H, CH<sub>3</sub>), 2.31 (s, 3H, N-CH<sub>3</sub>), 2.57-2.62 (m, 2H, aliphatic), 2.75-2.82 (m, 2H, aliphatic), 2.85-2.93 (m, 3H, aliphatic), 4.23-4.27 (d, *J*=15, 1H, H-15), 4.39-4.43 (d, *J*=15, 1H, H-15), 7.10-7.33 (m, 5H, aromatic), 7.29-7.33 (m, 2H, aromatic), 7.49-7.51 (d, *J*=7, 1H, aromatic), 7.92 (s, 1H, indole-*NH*). <sup>13</sup>C NMR: 400 MHz (CDCl<sub>3</sub>): δ 14.88 (CH<sub>3</sub>), 31.15 (5), 32.29 (9), 35.24 (15), 41.74 (N-CH<sub>3</sub>), 53.26 (6), 59.72 (8), 110.30 (13), 111.27 (9a), 118.09 (10), 119.03 (11), 120.97 (12), 126.49 (3), 126.76 (1), 129.57 (9b), 130.28 (2), 130.64 (4), 135.10 (14a), 135.25 (13a), 137.91 (4a), 141.07 (15a). HPLC retention time: 11.64 min (48.7%) and 22.10 min (48.99%) (column: Chiralcel OD; eluant: n-hexane/ ethanol 9:1; flow rate: 1.3 mL/min; UV detector λ=

220 nm). Anal. calcd. for  $C_{20}H_{20}N_2 \cdot xH_2O$ : C 78.40%, H 7.24%, N 9.14%; found C 78.00%, H 7.26%, N 9.06%.

The enantiomers were separated using a preparative Chiracel® OD column (Cellulose tris (3,5-dimethylphenylcarbamate). Eluent: hexane/ethanol 9:1; flow rate: 194 mL/min; temp.: 25 °C; UV-Detector  $\lambda$  = 220 nm. The isolation was accomplished by 1 mL injection of a solution of **5** (75 mg in 1 mL *n*-hexane/ethanol 1:1). Specific rotation of the first eluted enantiomer was  $[\alpha]_{546}^{rt} = 132^\circ$  (c 1,  $CHCl_3$ ), and for the second  $[\alpha]_{546}^{rt} = -128^\circ$  (c 1,  $CHCl_3$ ). Comparison of the specific rotation obtained for both enantiomers with that obtained for **(8S)-7,8-dimethyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecine (6) prepared by alternative synthetic route (Scheme 3)**  $[\alpha]_{546}^{rt} = 136^\circ$  (c 1,  $CHCl_3$ ), revealed that the first eluted enantiomer also bears an *S*-configuration at position 8.

**7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecine-8-carboxylic acid (16 and 17).** The same procedure for ring opening was applied. After evaporating the ammonia water was added and the obtained solution was carefully treated with 1N HCl, till the target amino acid precipitated as a creamy white solid (pH 6), which was filtered off and dried. White solid. Yield 60-67%; m.p. chars without melting at 220°C (analytical data see SI).

**[(8R)-7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecin-8-yl]methanol (24).** Purified by column chromatography dichloromethane/methanol (11:1). Yellowish white solid. Yield 67%, m.p. 83-85 °C (analytical data see SI).

**[(8S)-7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecin-8-yl]methanol (25).** Purified by column chromatography dichloromethane/methanol (11:1). Yellowish white solid. Yield 62.6%, m.p. 83-85 °C (analytical data see SI).

**General procedure for the reduction of the ester function:** To a suspension of lithium aluminium hydride (2 mmol) in dry THF was added a solution of respective ester (1 mmol) in dry THF and the reaction mixture was stirred at room temperature for 90 min (heated under reflux for 3 hrs for compounds **18** and **19**). Excess lithium aluminium hydride was quenched with methanol, the reaction mixture was filtered off and the filtrate was evaporated under reduced pressure.

**[(8RS)-7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecin-8-yl]methanol (18 and 19).** Purified by column chromatography dichloromethane/methanol (11:1). Creamy white solid. Yield 42%, m.p. 100-103°C.  $^1H$  NMR: 300 MHz ( $CDCl_3$ ):  $\delta$  2.24 (s, 3H, N- $CH_3$ ), 2.78-2.90 (m, 5H, aliphatic), 3.02-3.11 (m, 1H, aliphatic), 3.18-3.22 (m, 1H, aliphatic), 3.66-3.72 (m, 1H,  $\underline{CH_2}OH$ ), 3.76-3.81 (m, 1H,  $\underline{CH_2}OH$ ), 4.11-4.16 (d,  $J=15$ , 1H, H-15), 4.33-4.38 (d,  $J=15$ , 1H, H-15), 7.07-7.28 (m, 6H, aromatic), 7.34-7.35 (d,  $J=7$ , 1H, aromatic), 7.49-7.51 (d,  $J=8$ , 1H, aromatic), 7.98 (s, 1H, indole-*NH*). (Column: Teicoplanin T (150 x 4.6 mm i.d.); eluent: methanol-glacial acetic acid-triethylamine, (100:0.50:0.025); flow rate: 1.0 mL/min; UV detector  $\lambda$  = 282 nm). (Column: Teicoplanin T (150 x 4.6 mm i.d.); eluent: methanol-glacial acetic acid-triethylamine, (100:0.50:0.025); flow rate: 1.0 mL/min; UV detector  $\lambda$  = 282 nm).

**(8R)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-8-ylmethanol (20).** Crystallized from acetone/hexane giving yellowish crystals. Yield 82.6%, m.p. 198-200°C (analytical data see SI).

**(8S)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-8-ylmethanol (21).** Crystallized from acetone/hexane giving yellowish crystals. Yield 79.3%, m.p. 197-199°C (analytical data see SI).

**8-Carboxy-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-7-ium iodide (14 and 15).** To an ice cold-suspension of **12** or **13** (4.4 mmol) in 80 mL methanol was slowly added 27 mL 1N NaOH. The reaction mixture was stirred at 0 °C for 2 hours and then acidified with 1N HCl. Methanol was removed under reduced pressure and the remaining aqueous suspension was cooled in an ice bath for 30 minutes. The obtained pale yellow precipitate was filtered off and dried. Yield 71-77%; m.p.: chars without melting at 235°C. <sup>1</sup>H NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 2.99-3.59 (m, 4H, aliphatic), 3.44 (s, 3H, N<sup>+</sup>-CH<sub>3</sub>), 3.85-3.59 and 4.17-4.31 (m, 2H, aliphatic), 4.7 and 4.17-4.31 (m, 1H, H-8), 6.50-6.53 (2s, 1H, H-14b), 7.03-7.17 (m, 2H, aromatic), 7.35-7.62 (m, 6H, aromatic), 10.41 and 11.81 (2s, 1H, indole-NH).

**ACKNOWLEDGMENT** We thank Bärbel Schmalwasser, Petra Wiecha and Heidi Traber for skillful technical assistance in performing the pharmacological assays. Furthermore we would like to thank the DFG for the financial support of the project (EN 875/1-1).

**Supporting Information Available.** Physical and spectral data for some target and intermediary compounds; table of elemental analysis for key compounds; a detailed protocol for the pharmacological assays.

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# Supporting Information

## Synthesis and Dopamine Receptor Affinities of Racemic and Enantiopure Indolo[3,2-*f*][3]benzazecine Derivatives

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## I. Experimental synthetic procedures:

**General methods:** Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz). Elemental analyses were performed on a Hereaus Vario EL apparatus. TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific).

**$\alpha$ -Methyltryptamine (2).** prepared following the procedure described previously described in the literature<sup>1</sup>. A solution of indole-3-carboxaldehyde (4.35 g, 30 mmol) and ammonium acetate (1.2 g, 15 mmol) in a mixture of nitroethane (70 mL) and acetic acid (30ml) was heated at 110 °C for 8 h. The reaction mixture was cooled down to rt, whereupon an orange brown solid separated out. This was filtered off and the filtrate was concentrated to half its volume to precipitate the rest of nitropropenyl derivative. Collective yield: 3.66 g (65%); mp. 185-186 °C, reported 190-192 °C.  $^1\text{H}$  NMR: 250 MHz (DMSO- $d_6$ ):  $\delta$  2.48-2.50 (m, 3H,  $\text{CH}_3$ ), 7.12- 7.27 (m, 2H, aromatic), 7.48-7.51 (d,  $J=7$ , 1H, aromatic), 7.81-7.84 (d,  $J=7$ , 1H, aromatic), 7.99 (s, 1H, aromatic), 8.45 (s, 1H,  $\text{C}=\text{CH}$ ), 12.19 (s, 1H, indole-NH).

The nitropropenyl derivative (3.85 g, 19 mmol) was subsequently dissolved in dry THF (25 mL) and the solution was slowly added to a suspension of lithium aluminium hydride (3 g, 80 mmol) in dry THF (100 mL). The reaction mixture was heated under reflux for 6 h, cooled down to rt and then quenched with a saturated solution of potassium sodium tartarate. It was filtered off, and the filtrate was evaporated under vacuum leaving a yellowish oil, which solidified on standing. Yield 5.9 g (89%), mp. 90-82 °C, reported 97-100 °C.  $^1\text{H}$  NMR: 400 MHz ( $\text{CDCl}_3$ ):  $\delta$  1.19-1.21 (d,  $J=6$ , 3H,  $\text{CH}_3$ ), 1.56 (s, br, 2H,  $\text{NH}_2$ ), 2.66-2.72 (dd,  $J=14,8$ , 1H,  $\text{CH}_2$ ), 2.89-2.93 (dd,  $J=14,4$ , 1H,  $\text{CH}_2$ ), 3.30-3.33 (m, 1H, CH), 7.05 (s, 1H, aromatic), 7.12-7.15 (t,  $J=7$ , 1H, aromatic), 7.20-7.23 (t,  $J=7$ , 1H, aromatic), 7.37-7.39 (d,  $J=8$ , 1H, aromatic), 7.63-7.65 (d,  $J=8$ , 1H, aromatic), 8.27 (s, 1H, indole-NH).

**Methyl D- and L- tryptophanate (8 and 9).** Esterification of D- and L-tryptophan was carried out using  $\text{MeOH}/\text{SOCl}_2$ <sup>2</sup>. D- or L-tryptophan (5 g, 24.5 mmol) was added to a solution of thionyl chloride (4.25 mL, 58 mmol) in methanol (50 mL) and the resulting solution was heated under reflux for 18 h. After evaporation of the solvent, a white residue of hydrochloride salt was obtained, which was neutralized by a sodium carbonate solution and the ester was extracted with ethyl acetate (200 mL). The organic layer was dried over  $\text{MgSO}_4$  and evaporated under reduced pressure yielding a colourless oil, which solidified upon standing to a white solid. Yield: 4.9 g (92%), mp: 90-91 °C, reported 89 °C.  $^1\text{H}$  NMR: 250 MHz ( $\text{CDCl}_3$ ):  $\delta$  1.63 (s, 2H,  $\text{NH}_2$ ), 3.02-3.11 (dd,  $J=14,8$ , 1H,  $\text{CH}_2$ ), 3.26-3.43 (dd,  $J=14,5$ ,

1H, CH<sub>2</sub>), 3.72 (s, 3H, COOCH<sub>3</sub>), 3.82-3.87 (m, 1H, CH), 7.04-7.05 (d, *J*=, 1H, aromatic), 7.12-7.26 (m, 2H, aromatic), 7.33-7.37 (d, *J*=8, 1H, aromatic), 7.61-7.64 (d, *J*=8, 1H, aromatic), 8.28 (s, 1H, indole-NH).

**Methyl (8R)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinoline-8-carboxylate (10).** <sup>1</sup>H NMR: 250 MHz (CDCl<sub>3</sub>): δ 2.67-2.77 (m, 1H, aliphatic), 2.94-3.38 (m, 5H, aliphatic), 3.76 (s, 2H) and 3.89 (s, 1H) (COOCH<sub>3</sub>), 4.13-4.18 (dd, *J*=6, 1.5, 2/3H) and 4.22-4.29 (dd, *J*=11, 6, 1/3H) (H-8), 5.47 (s, 1/3H) and 5.62 (s, 2/3H) (H-14b), 7.07-7.42 (m, 7H, aromatic), 7.50-7.55 (m, 1H, aromatic), 7.73 (s, br, 1H, indole-NH). Anal. calcd. for C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub> x 0.75 H<sub>2</sub>O: C 72.92%, H 6.26%, N 8.10%; found C 73.24%, H 6.31%, N 8.10%.

**Methyl (8S)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinoline-8-carboxylate (11).** As expected, the <sup>1</sup>H NMR spectrum showed two sets of signals, corresponding to the two formed diastereomers. One of the diastereomers was obtained in a significantly higher amount (almost twofold excess) than the other. <sup>1</sup>H NMR: 250 MHz (CDCl<sub>3</sub>): δ 2.67-2.77 (m, 1H, aliphatic), 2.95-3.39 (m, 5H, aliphatic), 3.77 (s, 2H) and 3.89 (s, 1H) (COOCH<sub>3</sub>), 4.15-4.18 (dd, *J*=6, 2, 2/3H) and 4.23-4.30 (dd, *J*=11, 6, 1/3H) (H-8), 5.48 (s, 1/3H) and 5.65 (s, 2/3H) (H-14b), 7.07-7.42 (m, 7H, aromatic), 7.50-7.55 (m, 1H, aromatic), 7.71 and 7.76 (2s, 1H, indole-NH). Anal. calcd. for C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C 75.88%, H 6.06%, N 8.43%; found C 75.70%, H 5.89%, N 8.29%.

**8R-(Methoxycarbonyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-7-ium iodide (12).** <sup>1</sup>H NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 3.03-3.12 (m, 1H, aliphatic), 3.24-3.62 (m, 3H, aliphatic), 3.39 (s, 3H, N<sup>+</sup>-CH<sub>3</sub>), 3.84 (s, 3H, COOCH<sub>3</sub>), 3.84-4.04 (m, 1H, aliphatic), 4.10-4.12 (m, 1H, aliphatic), 5.15-5.20 (m, 1H, H-8), 6.33 and 6.36 (2s, 1H, 14b), 7.02-7.19 (m, 2H, aromatic), 7.36-7.39 (m, 2H, aromatic), 7.51-7.63 (m, 4H, aromatic), 10.35 (s, 1H, indole-NH). Anal. calcd. for C<sub>22</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>: C 55.71%, H 4.89%, N 5.91%; found C 55.56%, H 4.87%, N 5.60%.

**8R-(Methoxycarbonyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-7-ium iodide (13).** <sup>1</sup>H NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 3.04-3.12 m, 1H, aliphatic), 3.21-3.62 (m, 3H, aliphatic), 3.39 (s, 3H, N<sup>+</sup>-CH<sub>3</sub>), 3.84 (s, 3H, COOCH<sub>3</sub>), 3.84-4.04 (m, 1H, aliphatic), 4.10-4.12 (m, 1H, aliphatic), 5.15-5.20 (m, 1H, H-8), 6.33 and 6.36 (2s, 1H, 14b), 7.02-7.19 (m, 2H, aromatic), 7.36-7.39 (m, 2H, aromatic), 7.51-7.63 (m, 4H, aromatic), 10.35 (s, 1H, indole-NH).

**7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecine-8-carboxylic acid (16 and 17).** <sup>1</sup>H NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 2.29 (s, 3H, N-CH<sub>3</sub>), 2.61-3.03 (m, 6H, aliphatic), 3.27-3.31 (m, 1H, H-8), 4.17 (s, 2H, H-15), 6.88-7.01 (m, 2H, aromatic), 7.11-7.15 (mc, 3H, aromatic), 7.24-7.27 (d, *J*=8, 1H, aromatic), 7.36-7.44 (m, 2H, aromatic), 10.88 (s, 1H, indole-NH). HRMS 357.159 (calcd. for C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>: 357.158). <sup>13</sup>C NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 25.8 (5), 31.25 (9), 33.13 (15), 43.90 (N-

CH<sub>3</sub>), 53.55 (6), 68.41 (8), 108.51 and 108.54 (13), 110.95 and 111.00 (9a), 117.70 (10), 118.69 (11), 120.67 (12), 126.68 (3), 126.82 (1), 128.69 (9b), 130.68 (2), 130.85 (4), 135.31 and 135.47 (14a), 136.39 and 136.53 (13a), 138.51 (4a), 139.59 (15a), 173.63 (C=O). HRMS: 357.159 (calcd. C<sub>21</sub>H<sub>22</sub>NaN<sub>2</sub>O<sub>2</sub> 357.158).

**(8R)-8-(Hydroxymethyl)-7-methyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-a]isoquinolin-7-ium iodide (22).** <sup>1</sup>H-NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 3.00-3.18 (m, 1H, aliphatic), 3.23 and 3.27 (2s, 3H, N<sup>+</sup>-CH<sub>3</sub>), 5.56-5.60 and 5.64-5.68 (2t, *J*=5, 5H, H-8), 6.11 and 6.27 (2s, 1H, H-14b), 7.00-7.62 (m, 8H, aromatic), 10.23 and 11.55 (2s, 1H, indole-*NH*).

**[(8R)-7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecin-8-yl]methanol (24).**  
= -158.4° (c 1.26, CHCl<sub>3</sub>). <sup>1</sup>H-NMR: 400 MHz (CDCl<sub>3</sub>): δ 2.27 (s, 3H, N-CH<sub>3</sub>), 2.80-2.95 (m, 5H, aliphatic), 3.00-3.11 (m, 1H, aliphatic), 3.16-3.23 (m, 1H, aliphatic), 3.68-3.72 (m, 1H, CH<sub>2</sub>OH), 3.77-3.81 (m, 1H, CH<sub>2</sub>OH), 4.14-4.18 (d, *J*=15, 1H, H-15), 4.35-4.38 (d, *J*=15, 1H, H-15), 7.07-7.28 (m, 6H, aromatic), 7.34-7.36 (d, *J*=7, 1H, aromatic), 7.48-7.50 (d, *J*=8, 1H, aromatic), 7.91 (s, 1H, indole-*NH*). HPLC retention time: min (%) and min (%). (Column: Teicoplanin T (150 x 4.6 mm i.d.); eluent: methanol-glacial acetic acid-triethylamine, (100:0.50:0.025); flow rate: 1.0 mL/min; UV detector λ = 282 nm).

**[(8S)-7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-*f*][3]benzazecin-8-yl]methanol (25).**  
= 161° (c 1.25, CHCl<sub>3</sub>). <sup>1</sup>H-NMR: 400 MHz (CDCl<sub>3</sub>): δ 2.28 (s, 3H, N-CH<sub>3</sub>), 2.79-2.94 (m, 5H, aliphatic), 3.00-3.11 (m, 1H, aliphatic), 3.16-3.24 (m, 1H, aliphatic), 3.69-3.73 (m, 1H, CH<sub>2</sub>OH), 3.78-3.82 (m, 1H, CH<sub>2</sub>OH), 4.14-4.17 (d, *J*=15, 1H, H-15), 4.35-4.38 (d, *J*=15, 1H, H-15), 7.07-7.28 (m, 6H, aromatic), 7.34-7.36 (d, *J*=7, 1H, aromatic), 7.48-7.50 (d, *J*=8, 1H, aromatic), 7.88 (s, 1H, indole-*NH*). <sup>13</sup>C-NMR: 400 MHz (CDCl<sub>3</sub>): δ 25.86 (5), 32.18 (9), 33.00 (15), 40.98 (N-CH<sub>3</sub>), 53.71 (6), 63.28 (CH<sub>2</sub>OH), 64.18 (8), 110.43 (13), 110.63 (9a), 118.23 (10), 119.30 (11), 121.51 (12), 126.69 (3), 127.17 (1), 128.54 (9b), 130.26 (2), 130.69 (4), 133.39 (14a), 135.61 (13a), 136.93 (4a), 140.07 (15a). HPLC retention time: min (%) and min (%). (Column: Teicoplanin T (150 x 4.6 mm i.d.); eluent: methanol-glacial acetic acid-triethylamine, (100:0.50:0.025); flow rate: 1.0 mL/min; UV detector λ = 282 nm).

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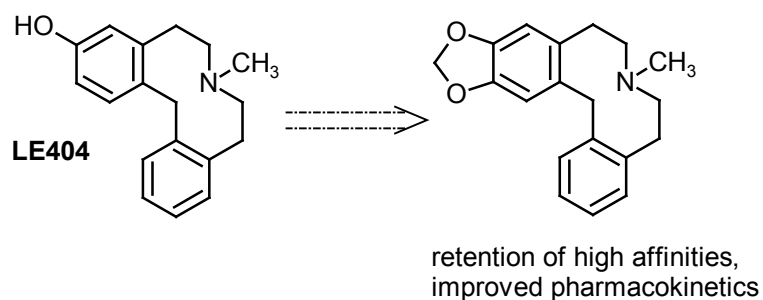
## Paper 5

### A Novel Nonphenolic Dibenzazecine Derivative with Nanomolar Affinities for Dopamine Receptors

*Dina Robaa, Shams EIDin AbulAzm, Jochen Lehmann, and Christoph Enzensperger*

*Chemistry and Biodiversity; manuscript accepted*

**Abstract:** A hexahydrodibenzo[*d,g*]azecine derivative, bearing a methylenedioxy moiety was prepared to further explore the SAR of the potent class of dibenzazecine-type dopamine receptor antagonists. Till now, all highly active dibenzazecines have been phenolic in nature; they all bear a 3-hydroxyl substituent. This phenolic nature is coupled with several drawbacks; specifically with respect to the poor pharmacokinetic profile. In this regard a methylenedioxy substituent would be more advantageous. The newly synthesized derivative showed similar affinities as the lead LE404, displaying nanomolar affinities for all dopamine receptor subtypes. Its dibrominated derivative, though exhibiting almost a fivefold decrease in affinities, still displayed nanomolar affinities. In the functional  $\text{Ca}^{2+}$  assay both novel derivatives were found to possess antagonistic properties at the dopamine receptors.



**Contribution:** Synthesis and characterization of all target and intermediary compounds, development of SAR and preparation of the manuscript.





# A Novel Non-phenolic Dibenzazecine Derivative with Nanomolar Affinities for Dopamine Receptors

By Dina Robaa<sup>a)</sup>, Shams ElDin AbulAzm<sup>b)</sup>, Jochen Lehmann<sup>a, c)</sup> and Christoph Enzensperger<sup>a\*)</sup>

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Dibenzazecines are a novel class of dopamine receptor antagonists, characterized by their high affinities as well as their tendency for D<sub>1</sub>-selectivity. Hitherto, the most active dibenzazecines were phenolic in nature; a 3-hydroxyl substituent was found to result in the highest affinities. However, the phenolic nature of these compounds mostly renders them unsuitable for *in vivo* application, due to the poor pharmacokinetic profile, imparted by the phenolic group. A novel dibenzazecine derivative was prepared, bearing a methylenedioxy moiety at the 2,3-position instead of the 3-hydroxyl group. The newly synthesized derivative **3** showed high affinities similar to the lead LE404, displaying nanomolar affinities for all dopamine receptor subtypes. Its dibrominated derivative **4**, though exhibiting almost a fivefold decrease in affinities, still displayed nanomolar ones for all dopamine receptors, except for D<sub>4</sub>. In a functional Ca<sup>2+</sup> assay both compounds **3** and **4** were found to possess antagonistic properties at the dopamine receptors.

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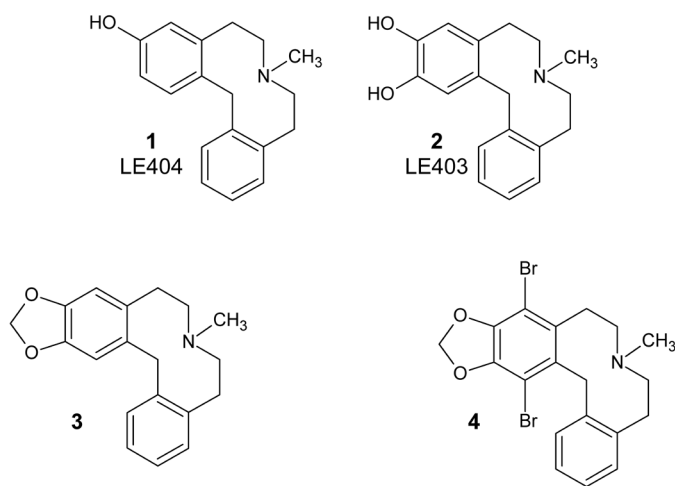
**Introduction:** Dopamine is a regulator of numerous physiological functions in the CNS and the periphery. Dysfunctions of the dopaminergic system have been implicated in the pathogenesis of several neurological disorders, including schizophrenia, Parkinson's disease, depression, attention-deficit hyperactivity disorder (ADHD) as well as drug and alcohol dependence. [1;2]

Dibenz[*d,g*]azecines are a novel class of dopamine receptor antagonists, which are distinguished by their new chemical structure as well as their high affinities for dopamine receptors, predominantly for the D<sub>1</sub> family. [3;4] SAR studies on this new class of compounds revealed that derivatives encompassing a dibenz[*d,g*]azecine moiety as the backbone structure and bearing a hydroxyl substituent at position 3 (e.g. compound **1**; LE404), display the highest affinities, specifically for human D<sub>1</sub> and D<sub>5</sub> receptors. [3-5] Changing the position of the hydroxyl group or replacing it by a methoxy function both led to decreased affinities. [4] Surprisingly, a 2,3-dihydroxy substitution pattern (compound **2**; LE403), which bears the most structural resemblance to dopamine, the natural ligand, showed ca. 100-1000 fold lower affinities than the monohydroxylated LE404). [3]

However, the high affinities of these dopamine antagonists are coupled with drawbacks. Phenols and especially dihydroxylated compounds tend to be chemically unstable. Due to their phenolic nature, these compounds are predisposed to poor pharmacokinetics (low bioavailability), which would restrict their utility for *in vivo* experiments and render them unsuitable for clinical development. Accordingly, our preliminary *in vivo* evaluations have shown that compound **1** (LE404) has a low oral bioavailability when administered to rats. (Schulze et al., J. Pharmacol. Exp. Therapeutics submitted)

Studies on other classes of phenolic dopamine antagonists, in particular SCH23390 and SCH39166, have revealed similar results. [6;7] Both compounds showed very low bioavailability besides a short duration of action. The latter fact has been attributed to the fast biotransformation of these phenolic compounds, occurring mainly by glucuronidation at the phenolic function. [6;7] Analogues lacking

this phenolic group have indeed been found to exhibit better pharmacokinetics and longer duration of action, which is more advantageous for *in vivo* application. [8]



**Figure 1.** The 3-hydroxybenzazecine derivative LE404, the inactive 2,3-dihydroxy analogue LE403, and the target methylenedioxy analogues **3** and **4**

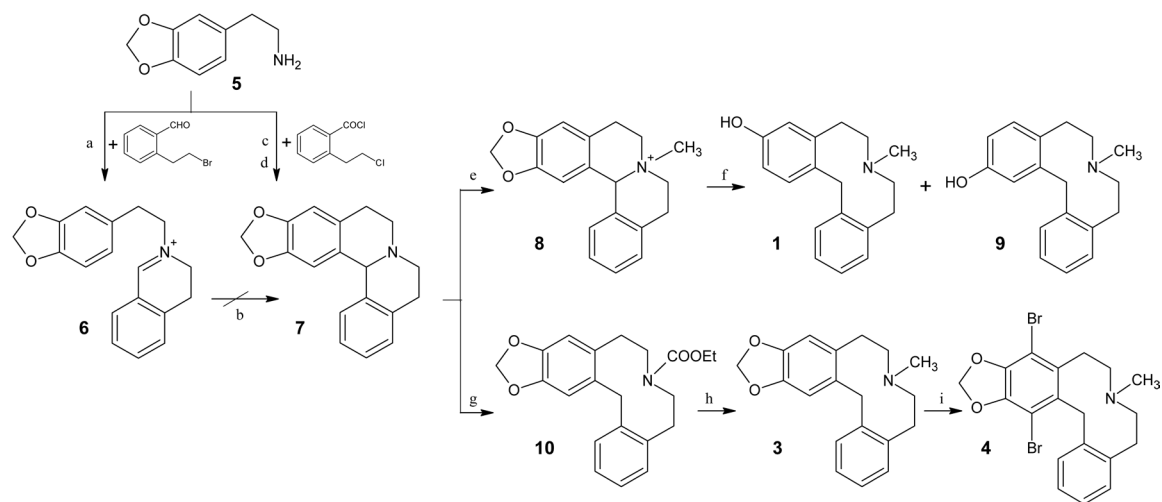
Goal of the present study was to prepare a dibenz[*d,g*]azecine where positions 2 and 3 are connected through a methylenedioxy moiety. This was appealing for the following reasons.

- Our previous studies have shown that the nature, number and position of the substituents at the benzene ring significantly affect the binding affinities. It was hence interesting to investigate the influence of this spatially rather confined group on the affinities. A methylenedioxy moiety, shares similar electronic properties with the 2,3-dihydroxy and -dimethoxy substituents, but is spatially distinct from all previously examined substitution patterns.
- Compared with the previously synthesized phenolic dibenzazecines the target compound **3** is presumably chemically more stable and conceivably of better pharmacokinetics.

## Results:

**Chemistry:** As outlined in Scheme 1, the target dibenzazecine **3** was obtained through its pentacyclic precursor quinolizine **7**. Our previously established procedure, where the amine was reacted with 2-(2-bromoethyl)benzaldehyde in the presence of trifluoroacetic acid, [9] only yielded the intermediary isoquinolinium salt **6** and not the expected quinolizine **7**. Trials to cyclise the obtained isoquinolinium salt by *Pictet Spengler* cyclization using 6N HCl were unsuccessful. The quinolizine **7** was thus achieved through the reaction of the precursor amine **5** with 2-(chloroethyl)benzoyl chloride, and the obtained intermediate was directly cyclised using *Bischler-Napieralski* conditions and subsequently reduced. Conversion of the quinolizine **7** to the target dibenzazecine **3** was similarly knotty. Conventionally at that step, the quinolizine derivative (**7**) was quaternized with methyl iodide and the obtained quaternary salt was cleaved under *Birch* conditions to the corresponding azecine. In this case, reduction of the quaternary salt **8** with sodium in liquid ammonia gave a 1:1 mixture of two different compounds having the same molecular weight. These substances were identified by means of GCMS as the phenolic azecines **1** (LE404) and **9**, which was confirmed by spiking the GCMS with reference samples of **1** and **9**, both available in our lab from previous work. The phenomenon of the cleavage of the methylenedioxy moiety under various reductive conditions has been formerly described. [10;11] For instance, reduction under *Birch* conditions (sodium and liquid ammonia) was found to result in the cleavage of methylenedioxy derivatives, yielding the respective *p*-phenolic compounds. [10] It has

been suggested that the nature of the substituent positioned opposite to the methylenedioxy moiety directs the site of cleavage, which leads to the formation of either the *m*- or *p*-phenolic compound. In our case, however, cleavage of the methylenedioxy group occurred at two different positions and not at only one position, as observed with reported methylenedioxy derivatives. [10] This of course may be attributed to the fact that both substituents lying across the methylenedioxy group exert equal effects.



**Scheme 1.**

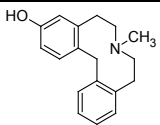
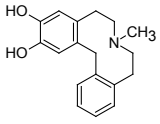
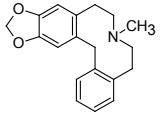
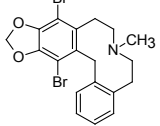
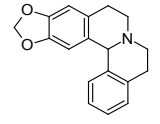
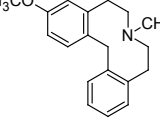
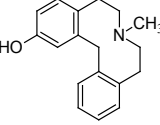
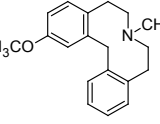
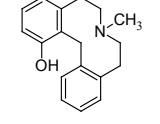
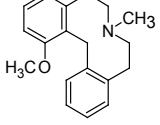
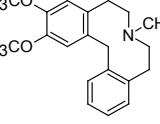
**Reagents and conditions:** (a) TFA, dioxane, reflux, 12 h; (b) 6N HCl, reflux, 10 h; (c) NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 90 min; (d) POCl<sub>3</sub>/MeCN (1:2), reflux, 36h; NaBH<sub>4</sub>, MeOH, reflux, 2h; (e) MeI, acetone; (f) Na<sup>0</sup>, liq. NH<sub>3</sub>, -40°C, 10min; (g) ClCOOEt, dry THF, -65°C, 4h, then NaCNBH<sub>4</sub>, -65°C to rt; (h) LiAlH<sub>4</sub>, dry THF, reflux, 2h; (i) Br<sub>2</sub>, AlCl<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 10h.

To accomplish ring opening and avoid degradation of the methylenedioxy function, the quinolizine **7** was first converted to the carbamate **10** according to literature [12], which was subsequently reduced with LAH to give the target compound **3**. This azecine was further dibrominated, to examine the impact of the additional bromine atoms on the affinities. Unfortunately, the monobrominated derivatives could not be obtained, even when equimolar amounts of bromine were used.

### Pharmacology:

The target compounds **3** and **4** as well as the precursor quinolizine **7** were screened for their affinities for the human cloned dopamine receptor subtypes D<sub>1</sub>, D<sub>2L</sub>, D<sub>3</sub>, D<sub>4.4</sub>, and D<sub>5</sub>. These receptors were stably expressed in HEK293 or CHO cells; [<sup>3</sup>H]SCH23390 and [<sup>3</sup>H]spiperone were used as radioligands at the D<sub>1</sub>-like and D<sub>2</sub>-like receptors, respectively. *K<sub>i</sub>* values are given in nanomolar units (Table 1). In addition, the compounds were tested in an intracellular Ca<sup>2+</sup> assay, to determine their functionality at the D<sub>1</sub> and D<sub>2</sub> receptors. For this purpose, HEK293 cells, stably expressing the respective D-receptor were loaded with a fluorescent dye, and after preincubation with rising concentrations of the test compound, an agonist (SKF 38393 for D<sub>1</sub> and quinpirole for D<sub>2</sub>) was injected and the Ca<sup>2+</sup>-induced fluorescence was measured with a microplate reader. The ability of the test compound to suppress the agonist-induced Ca<sup>2+</sup> influx is an indication of antagonistic or inverse agonistic properties at the receptor. Both the radioligand binding assay and the Ca<sup>2+</sup> assay have been described in detail. [9]

**Table 1.** Affinities ( $K_i$ , nM) For Human  $D_1$ - $D_5$  Receptors, Determined by Radioligand Binding Experiments

Compounds		$K_i$ (nM)				
		HEK $D_1$	HEK $D_{2L}$	HEK $D_3$	CHO $D_{4,4}$	HEK $D_5$
	<b>1</b> <b>(LE404)</b>	0.39±0.22 <sup>a</sup>	17.5±1.5 <sup>a</sup>	47.5±24 <sup>b</sup>	11.3±1.0 <sup>a</sup>	1.5±0.5 <sup>a</sup>
	<b>2</b> <b>(LE403)</b>	341±41 <sup>a</sup>	>5000 <sup>a</sup>	n.d.	165±12 <sup>a</sup>	1078±42 <sup>a</sup>
	<b>3</b>	3.6±1 <sup>*</sup>	5.5±1.2 <sup>*</sup>	20.6±5.5 <sup>*</sup>	46.0±9.9 <sup>*</sup>	0.5±0.01 <sup>*</sup>
	<b>4</b>	35.0±14.5 <sup>*</sup>	30.0±19.6 <sup>*</sup>	38.9±1.1 <sup>*</sup>	2760 ± 552 <sup>*</sup>	4.7±1.9 <sup>*</sup>
	<b>7</b>	>10000	>10000	>10000	>10000	>10000
	<b>11</b>	28.5±9.7 <sup>b</sup>	13.0±9.00 <sup>b</sup>	75.7±7.30 <sup>b</sup>	43.4±13.3 <sup>c</sup>	54.0±20 <sup>c</sup>
	<b>12</b>	8.9±0.8 <sup>b</sup>	36.9±27.8 <sup>b</sup>	296±60.0 <sup>b</sup>	913±79.0	8.92±0.82
	<b>13</b>	82.0±30 <sup>b</sup>	62.0±5.00 <sup>b</sup>	151±57.5 <sup>b</sup>	5987±675	14.4±8.5
	<b>14</b>	8.70±2.00 <sup>b</sup>	84.1±2.75 <sup>b</sup>	215±94 <sup>b</sup>	202±73.5	8.6±3.9
	<b>15</b>	7.6±1.5 <sup>b</sup>	164±12 <sup>b</sup>	1833±292 <sup>b</sup>	390±160	10.5±2.8
	<b>16</b>	509±51	>5000	n.d.	2514±101	2610±120

<sup>a</sup> Values from ref. [3]. <sup>b</sup> Values from ref. [4]. <sup>c</sup> Values from ref. [14]. <sup>\*</sup>  $K_i$  values are the means of two experiments; performed in triplicate ± SEM.

## Discussion:

The newly synthesized dibenzazecine derivative **3** proved to be a highly active dopamine antagonist (functional  $\text{Ca}^{2+}$  assay), showing comparatively high affinities at the different dopamine receptors as the lead compound **1** (LE404), all lying in the nanomolar range. The  $\text{D}_1$  selectivity profile, encountered with most members of this class of dopamine antagonists, was however lost. Compound **3** displayed equal affinities for the  $\text{D}_1$  and  $\text{D}_2$  subtypes and showed the highest selectivity for the  $\text{D}_5$  receptor ( $\text{D}_1/\text{D}_5 \approx 7$ ). With respect to the affinities, compound **3** is superior to previously synthesized 1- and 2-monohydroxylated derivatives (**14** and **12**) and the 1-, 2- and 3-monomethoxylated dibenzazecine derivatives (**15**, **13** and **11**) and similarly active to the 3-hydroxylated compound **1**. Compared with the 2,3-dihydroxy (**2**) and the -dimethoxy derivative (**16**) it showed a tremendous improvement in affinities; almost 100 fold increase in the affinity for  $\text{D}_1$  and 1000 fold for  $\text{D}_2$  and  $\text{D}_5$  was observed. The dibromo derivative **4** displayed the highest affinity for the  $\text{D}_5$  receptor, for the  $\text{D}_1$ ,  $\text{D}_2$  and  $\text{D}_3$  it showed similar but somewhat lower affinities while the  $\text{D}_4$  affinities were strongly decreased.

Previous SAR studies have already indicated that a hydroxyl group at position 3 of the dibenzazecine ring is crucial for high affinities. It presumably interacts through H-bond formation with a serine residue (Ser 5.46 or Ser 5.50) in the receptor binding pocket, as already observed with other dopamine antagonists at the  $\text{D}_2$  receptor. [13]

Derivatives bearing a methoxy substituent especially at position 2 or 3 showed significantly decreased affinities. This could be attributed to the fact that a methoxy group, which rotates freely, is spatially too bulky for the receptor binding site. A 2,3-dimethoxy substitution pattern requires much more space due the free internal rotation of both methoxy groups, hence resulted in a pronounced decrease in affinities. Conversely, a methylenedioxy substituent, which requires less space due to its restricted rotation, showed similarly high affinities as the highly active 3-hydroxy derivative **1**. From our observations it can be drawn that the phenolic compounds rather act as H-acceptor than as H-donor.

Taken together, a methylenedioxy moiety placed at the 2,3-position of the dibenzazecine ring is not only favoured in terms of chemical stability and pharmacokinetics but also in regards to the binding affinities to dopamine receptors.

## Experimental:

**General methods.** Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Purities of the compounds were determined by elemental analysis, performed on a Hereaus Vario EL apparatus. All values for C, H and N were found to be within  $\pm 0.4$ . All compounds showed >95% purity.

### 2-[2-(1,3-Benzodioxol-5-yl)ethyl]-3,4-dihydroisoquinolinium trifluoroacetate (**6**)

A solution of 2-(1,3-benzodioxol-5-yl)ethylamine (**5**) (1.65 g, 10 mmol), 2-(2-bromoethyl)benzaldehyde [15] (3.2 g, 15 mmol) and trifluoroacetic acid (1.7 g, 15 mmol) in dioxane (40 ml) was heated under reflux for 12 hours. The isoquinolinium salt, and not the expected quinolinizinium salt, separated as a yellow solid. This was filtered off, washed with dioxane, then diethylether and dried (1.2 g, 36%). M.p. 214-216°C.  $^1\text{H}$ -NMR: 250 MHz ( $\text{MeOD}-d_4$ ):  $\delta$  3.16-3.22 (t,  $J = 7$ , 2H, 4), 3.25-3.31 (t,  $J = 8$ , 2H, ethylene-2), 4.10-4.16 (t,  $J = 8$ , 2H, ethylene-1), 4.24-4.30 (t,  $J = 7$ , 2H, 3), 5.93 (s, 2H,  $\text{OCH}_2\text{O}$ ), 6.74-6.81 (mc, 2H, aromatic), 6.89 (s, 1H, aromatic), 7.48-7.56 (m, 2H, aromatic), 7.73-7.83 (m, 2H, aromatic), 8.91 (s, 1H,  $\text{N}^+=\text{CH}$ ).

### 5,8,9,14b-Tetrahydro-6H-[1,3]dioxolo[4,5-g]isoquino[1,2-a]isoquinoline (7)

To an ice-cooled solution of 2-(1,3-benzodioxol-5-yl)ethylamine (**5**) (2.97 g, 18 mmol) in dichloromethane (30 ml) was added triethylamine (5.45 g, 54 mmol) followed by a solution of 2-(chloroethyl)benzoylchloride (4.02 g, 19.8 mmol) in dichloromethane (10 ml). The reaction mixture was stirred at room temperature for 90 min, subsequently washed with 10% HCl (2x30 ml) followed by brine (30 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure yielding a brown oil. This crude amide was dissolved in a mixture of POCl<sub>3</sub>/acetonitrile (15 ml/30 ml) and the reaction mixture was heated under reflux for 36 h. The solvents were then removed under reduced pressure and the remaining POCl<sub>3</sub> was removed by washing with petroleum ether (40-60) followed by diethylether. The residue was dissolved in methanol (150 ml) without prior purification, then NaBH<sub>4</sub> (4 g) was carefully added to the ice-cooled solution. The reaction mixture was heated under reflux for 2 h, the solvent was subsequently removed under reduced pressure and the residue treated with water (100 ml) and extracted with diethylether (2x100ml). The collected organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The obtained oil was purified by column chromatography (EtOAc/MeOH, 9:1) yielding a yellowish white oil (0.52 g, 10%) which solidified on standing. M.p. 122-123°C. <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): δ 2.74-2.94 and 3.04-3.26 (2m, 8H, 5, 6, 8 and 9), 5.00 (s, 1H, 14b), 5.89 and 5.91 (2s, 2H, 12), 6.61 (s, 1H, 10), 6.69 (s, 1H, 14), 7.11-7.26 (m, 4H, 1-4). GC-MS: m/z: 278 (100%), 250 (25%), 191 (8%), 178 (81%), 165 (16%), 148 (11%), 130 (11%), 115 (24%), 103 (19%), 89 (23%), 77 (39%), 63 (30%). Anal. calcd. for C<sub>18</sub>H<sub>17</sub>NO<sub>2</sub> x 0.1 EtOAc: C 76.79%, H 6.23%, N 4.86%. Found: C 76.73%, H 6.19%, N 4.94%.

### Ethyl 5,8,9,15-tetrahydrobenzo[d][1,3]benzodioxolo[5,6-g]azecine-7-carboxylate (10)

A stirred solution of the quinolizine **7** (0.78 g, 2.7 mmol) in dry THF (50 ml) was cooled in methanol/dry ice at -65°C. While keeping the reaction mixture under nitrogen, ethyl chloroformate (1.63 g, 15 mmol) was added and stirring was continued for 4 h. Then a solution of sodium cyanoborohydride (0.59 g; 9.4 mmol) in dry THF (10 ml) was slowly added at -65°C and the reaction mixture was stirred overnight while allowing it to reach room temperature. It was subsequently treated with 2N NaOH (120 ml), the THF layer was separated, washed with brine, and finally the organic layer was evaporated under reduced pressure to give a yellow waxy solid. The obtained product was purified using column chromatography (EtOAc/n-hexane, 1:2) yielding a white solid (0.65 g, 69%). M.p. 110°C <sup>1</sup>H-NMR: 400 MHz (CDCl<sub>3</sub>): δ 0.74-0.80 and 0.94-1.00 (2t, *J* = 11, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 2.33-2.37 and 2.46-2.50 (2t, *J* = 9, 2H, 5), 2.86-2.94 (m, 2H, 9), 3.40-3.57 (m, 4H, 6,8), 3.79-3.78 and 3.82-3.90 (2q, *J* = 11, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 3.98 (s, 2H, 15), 5.89 (s, 2H, 12), 6.57, 6.61, 6.64 and 6.74 (4s, 2H, 10, 14), 7.03-7.26 (m, 4H, 1-4). <sup>13</sup>C-NMR: 400 MHz (CDCl<sub>3</sub>): δ 14.35 and 14.33 (OCH<sub>2</sub>CH<sub>3</sub>), 32.63 and 33.09 (5), 33.82 and 34.17 (9), 37.94 and 38.44 (15), 51.74 and 51.86 (6), 52.85 and 53.69 (8), 60.80 and 60.90 (OCH<sub>2</sub>CH<sub>3</sub>), 100.78 (12), 126.23, 126.47, 126.68 and 126.75 (2, 3), 129.97 and 130.92 (1), 131.06 and 132.08 (4), 132.39 and 132.77 (9a), 133.46 and 133.67 (14a), 139.53 and 139.60 (4a), 140.00 and 140.34 (15a), 146.07, 146.26 and 146.56 (10a, 13a), 156.38 (C=O). GC-MS: m/z: 353 (28%), 280 (9%), 248 (31%), 237 (16%), 220 (72%), 207 (12%), 191 (17%), 178 (70%), 165 (100%), 152 (47%), 139 (19%), 128 (30%), 115 (64%), 104 (70%), 91 (41%), 77 (55%), 65 (41%). Anal. calcd. for C<sub>21</sub>H<sub>23</sub>NO<sub>4</sub>: C 71.37%, H 6.56%, N 3.96%. Found: C 71.17%, H 6.58%, N 3.87%.

### 7-Methyl-5,6,7,8,9,15-hexahydrobenzo[d][1,3]benzodioxolo[5,6-g]azecine (3)

A solution of compound **10** (0.8 g, 2.3 mmol) in dry THF (20 ml) was slowly added to an ice-cooled, stirred suspension of lithium aluminium hydride (0.25 mg, 9 mmol) in dry THF (75 ml) while keeping the reaction under nitrogen. After the addition was completed, the reaction mixture was heated under reflux for 2 h. It was then cooled in an ice bath, and the excess of unreacted lithium aluminium hydride was quenched by careful addition of saturated potassium sodium tartarate solution until no further H<sub>2</sub> evolved. The resulting suspension was then filtered off and the filtrate evaporated under

reduced pressure, to give a white waxy solid **a** (0.65 g, 97%). The obtained product was purified by column chromatography (EtOAc/MeOH, 9:1). M.p. 70-71°C. <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): δ 2.26 (s, 3H, N-Me), 2.64-2.71 (mc, 8H, 5, 6, 8, 9), 4.35 (s, 2H, 15), 5.88 (s, 2H, 12), 6.56 (s, 1H, 10), 6.75 (s, 1H, 14), 7.04-7.33 (m, 4H, 1-4). <sup>13</sup>C-NMR: 250 MHz (CDCl<sub>3</sub>): δ 34.46 and 34.48 (5, 9), 38.06 (15), 46.92 (N-Me), 60.70 and 60.74 (6, 8), 100.66 (12), 109.99 (10, 14), 126.10 and 126.29 (2, 3), 130.52 and 130.89 (1, 4), 133.63 and 133.88 (9a, 14a), 141.02 and 141.09 (4a, 15a), 145.78 and 145.84 (10a, 13a). GC-MS: m/z: 295 (12%), 237 (30%), 223 (38%), 207 (10%), 190 (32%), 178 (60%), 165 (100%), 152 (39%), 139 (16%), 128 (21%), 115 (55%), 103 (33%), 89 (43%), 71 (60%), 63 (41%). Anal. calcd. for C<sub>19</sub>H<sub>21</sub>NO<sub>2</sub> x 0.1 EtOAc: C 76.60%, H 7.22%, N 4.60%. Found: C 76.78%, H 7.28%, N 4.57%.

#### **10,14-Dibromo-7-methyl-5,6,7,8,9,15-hexahydrobenzo[d][1,3]benzodioxolo[5,6-g]azecine (4)**

To a suspension of compound **3** (0.15 g, 0.5 mmol) and anhydrous AlCl<sub>3</sub> (0.03 g, 0.23 mmol) in dichloromethane (4 ml) was slowly added a solution of bromine (0.4 g, 2.5 mmol) in dichloromethane (2 ml). After 10 h of stirring at room temperature, the reaction mixture was poured onto a saturated solution of sodium metabisulfite (50 ml) and extracted twice with dichloromethane (2x15 ml). It was washed with saturated NaHCO<sub>3</sub> solution (2x50 ml) and twice with water (2x50 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure, leaving a brownish solid, which was crystallized from methanol giving yellow crystals. (0.15 g, 67.6%). M.p. 135-136°C. <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>): 2.35 (s, 3H, N-Me), 2.65-2.78 (m, 8H, 5, 6, 8, 9), 4.86 (s, 2H, 15), 6.09 (s, 2H, 12), 7.03-7.26 (m, 4H, 1-4). <sup>13</sup>C-NMR: 250 MHz (CDCl<sub>3</sub>): δ 32.71 (5), 34.88 (9), 37.95 (15), 46.54 (N-Me), 58.89 (6), and 60.79 (8), 101.26 (12), 104.23 and 104.43 (10, 14), 126.24 (2), 126.43 (3), 130.15 (4), 130.79 (1), 135.17 (9a), 135.59 (14a), 139.31 (4a), 141.35 (15a), 144.20 and 144.27 (10a, 13a). Anal. calcd. for C<sub>19</sub>H<sub>19</sub>Br<sub>2</sub>NO<sub>2</sub>: C 50.36%, H 4.23%, Br 35.26%, N 3.09%. Found: C 50.20%, H 4.29%, Br 35.08%, N 2.96%.

#### **Acknowledgment**

We thank Bärbel Schmalwasser, Petra Wiecha and Heidi Traber for skillful technical assistance in performing the pharmacological assays. Furthermore we would like to thank the DFG for the financial support of the project (EN 875/1-1).

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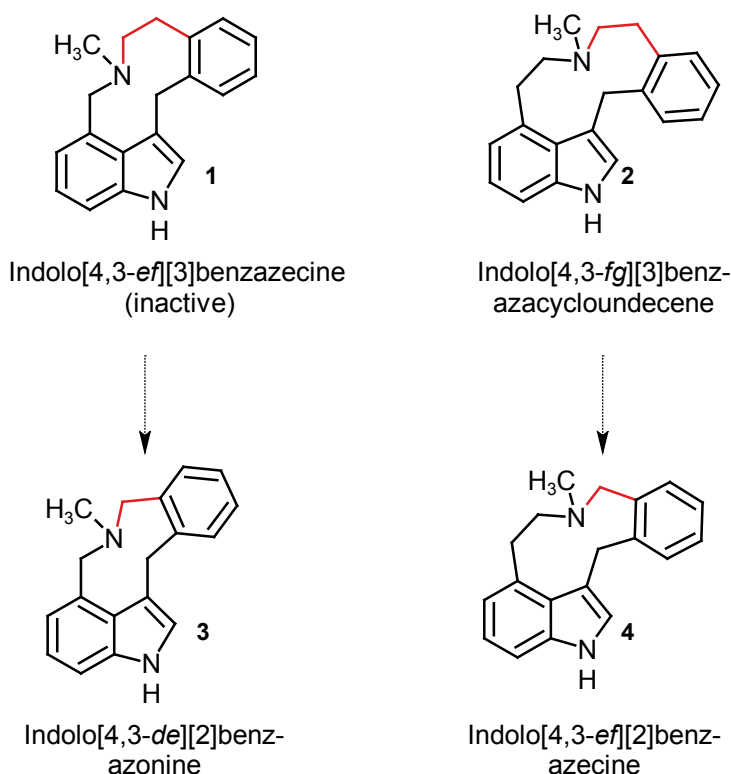
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## 4. Unpublished results

### 4.1. Trials to synthesize the [4,3-*ef*][2]benzazecine and indolo[4,3-*de*][2]benzazonine derivatives **3** and **4**

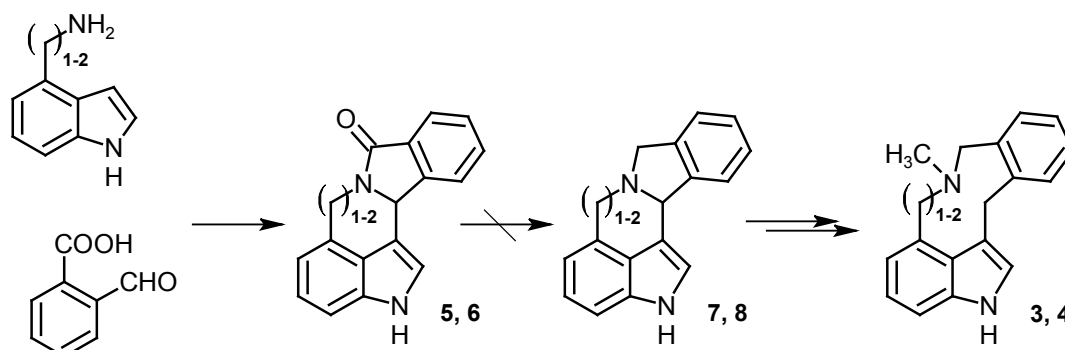
The indolo[4,3-*ef*][3]benzazecine and indolo[4,3-*fg*][3]benzazaundecene derivatives **1** and **2** showed a strong discrepancy in activities (**paper 1**). Compound **1** containing a ten-membered central ring displayed no notable affinities for the dopamine receptors, while **2** encompassing an eleven-membered central ring was similarly active to the lead compound LE300. Therefore, it was interesting to examine the affinities of other ring size-reduced analogues (**figure 4.1**) bearing the same fusion pattern.



**Figure 4.1:** Ring size-reduced analogues of the active indolo[4,3-*fg*]benzazaundecene and the inactive indolo[4,3-*ef*]benzazecine derivatives.

To obtain the target derivatives **3** and **4**, the precursor lactams **5** and **6** were prepared following a previously reported procedure used for the synthesis of analogous lactams<sup>106</sup>. For this purpose, the respective amines (4-aminoethyl- and 4-aminomethylindole) were reacted with 2-formylbenzoic acid in a mixture of ethanol and water in the presence of sulfuric acid. (**Figure 4.2**) Diverse trials to reduce the obtained lactams to the corresponding quinolizine and indolizine derivatives **7** and **8** were all in vain; the unreacted lactams were ultimately

recovered. These trials included reduction with lithium aluminum hydride, alane (a mixture of lithium aluminum hydride and aluminum chloride) as well as borane. (**Figure 4.2**)

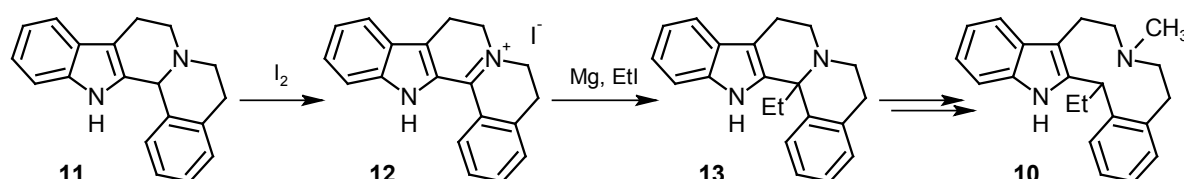


**Figure 4.2:** Synthetic route for the synthesis of indolo[4,3-ef][2]benzazecine and indolo[4,3-de][2]benzazonine.

## 4.2. LE300 substituted at the methylene bridge

Small substituents (methyl and ethyl) at the indole nitrogen of LE300 have been found to be either advantageous or well tolerated with regard to the biological activity (**paper 2**). It was thus interesting to similarly substitute the neighboring methylene group connecting both aromatic moieties in order to test the effect of small substituents placed near the indole nitrogen. We have already reported on the 15-methylated derivative **9**<sup>112</sup> (**table 4.1**), here it was planned to further derivatize that position with an ethyl and a methoxy group.

### 4.2.1. Substitution of LE300 with an ethyl group at the methylene bridge

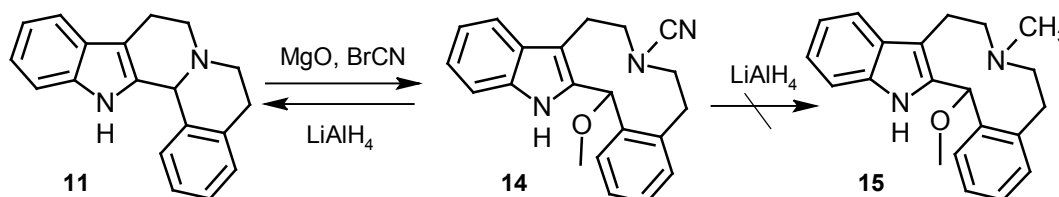


**Figure 4.3:** Scheme for the synthesis of an LE300 derivative substituted at the methylene bridge.

The target 15-ethylated LE300 (**10**) was obtained in a four-step-procedure. First the quinolizine **11** was oxidized by iodine to the iminium salt **12**, which was treated by the Grignard reagent from Mg and ethyl iodide to give the 14b-ethylated quinolizine **13**. The latter was quaternized with methyl iodide and acetone and subsequently reduced under Birch conditions to afford the desired benzindoloazecine derivative **10**. (**Figure 4.3**)

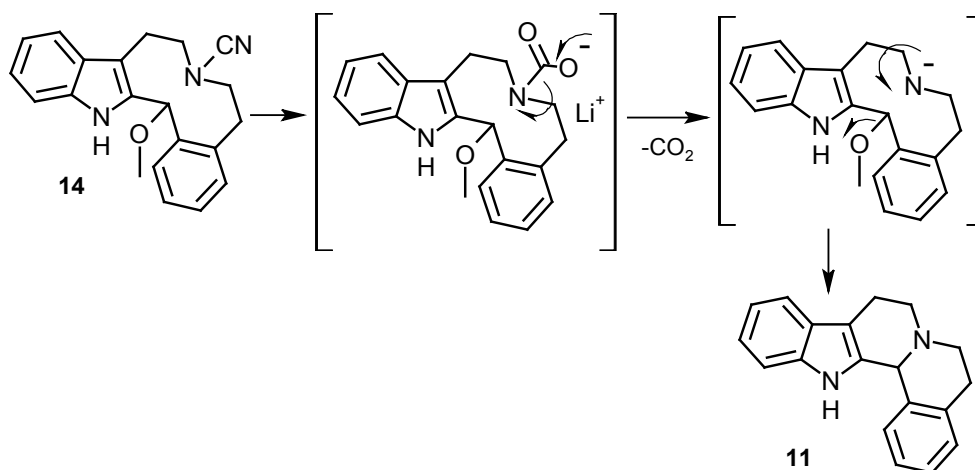
#### 4.2.2. Trials to synthesize an LE300 derivative bearing a methoxy group at the methylene bridge

First, the 7-cyano-15-methoxybenzindoloazecine derivative **14** was prepared by reaction of the quinolizine **11** with cyanogen bromide and methanol according to a procedure described by Browne for the synthesis of analogous benzothiophene and thiophene derivatives<sup>113</sup>. (Figure 4.4)



**Figure 4.4:** Synthetic approach for the synthesis of an LE300 derivative substituted with a methoxy group at the methylene bridge.

Applying the reported procedure for the conversion of the cyano- (**14**) to the corresponding methylazecine **15**, namely by reduction with lithium aluminum hydride<sup>113</sup>, unexpectedly led to the recovery of the starting quinolizine derivative **11**. (Figure 4.4)



**Figure 4.5:** Speculated mechanism for the conversion of the cyanoazecine **14** into the starting quinolizine **11** after reduction with lithium aluminum hydride.

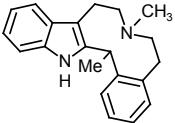
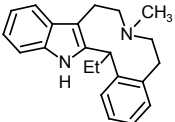
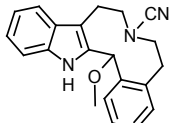
This presumably took place through the formation of the intermediary carbamic acid derivative, which dissociates by elimination of carbon dioxide. The formed intermediate, bearing negatively charged nitrogen, is unstable and thus directly eliminates a methoxide moiety to give the unsubstituted quinolizine derivative **11**. (Figure 4.5) It is however incomprehensible why this elimination took place in case of the cyanobenzindoloazecine derivative and not as reported<sup>113</sup> with related benzothiophene and thiophene derivatives.

### 4.2.3. Affinities for the dopamine receptors

Results of the radioligand binding experiments (**table 4.1**) revealed that substitution at position 15 of the benzindoloazecine scaffold is not well tolerated; both ethyl and methyl substitution led to a decrease in affinities, most pronouncedly for the D<sub>3</sub>-receptor.

The cyanoazecine derivative **14** was found to be devoid of affinities for all dopamine receptors ( $K_i > 10000$  nM), most likely due to the decreased (or nonexistent) basicity of the central nitrogen. (For  $K_i$  values see **Table 4.1**)

**Table 4.1:** Affinities ( $K_i$ , nM) of derivatives substituted at the methylene bridge.

Compounds	$K_i$ (nM), (Radioligand binding experiments)				
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
<b>9</b> <sup>112</sup> 	49 ± 7	200 ± 32	>10000	533 ± 45	64 ± 28
<b>10</b> 	116 ± 66	725 ± 102	>10000	298	21.7 ± 3
<b>14</b> 	>10000	>10000	n.d.	>10000	>10000

### 4.3. 8-Substituted benzindoloazecines

#### 4.3.1. Racemized benzindoloazecines derived from D- and L-tryptophan

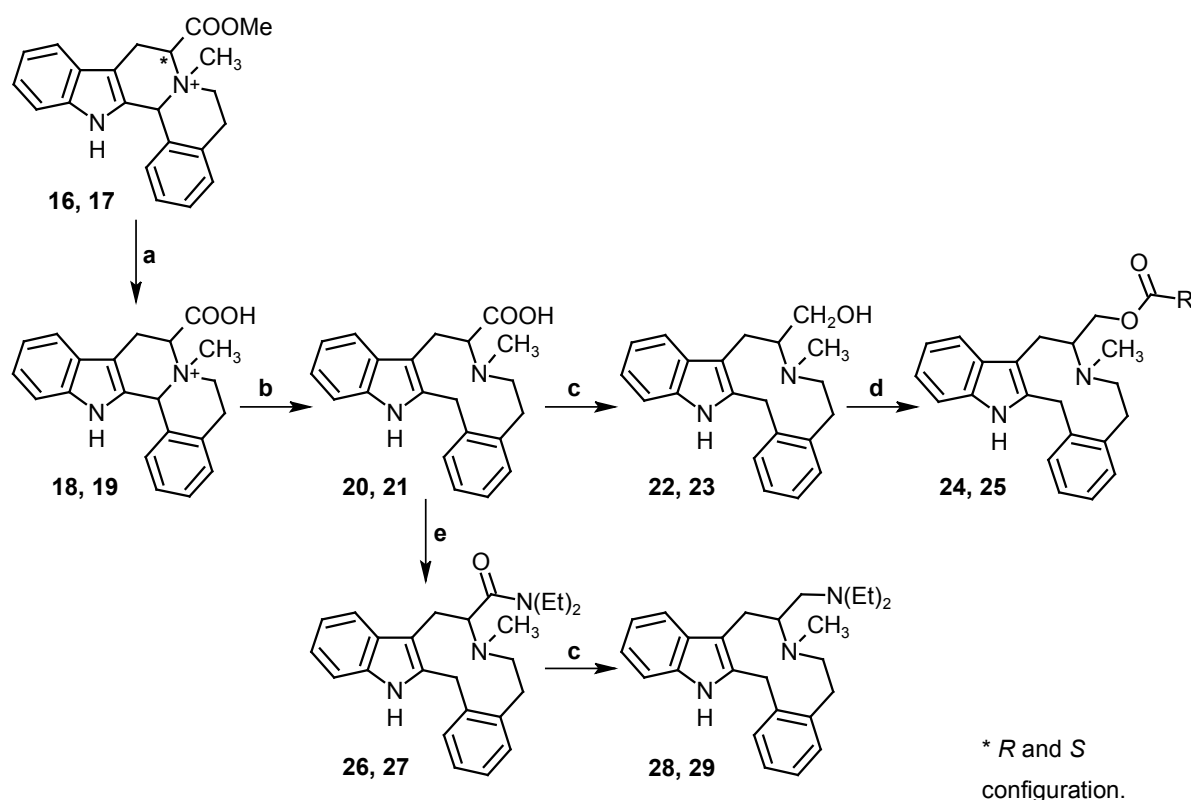
In our attempts to synthesize LE300 derivatives substituted at position 8, we set to prepare benzindoloazecines starting from D- and L-tryptophan and to further derivatize them. Goal was not only to prepare derivatives bearing different functional groups in order to study the effect of the various substituents at that position, but also to obtain them in an enantiomerically pure form with the purpose of examining the influence of the stereochemistry on the affinities for the dopamine receptors. As described in **paper 4** enantiopure azecines substituted at position 8 with methyl and hydroxymethyl groups were obtained, and the affinities of the *R*- and *S*-enantiomers were found to be markedly different. Several other azecines substituted at position 8 with various functional groups, including carboxy (**paper 4**), acyl, amide and amine functions, were successfully prepared. (**Figure 4.6**) Although enantiopure starting materials were used, various findings indicated that the obtained products were racemized and not enantiopure as expected. In the following part, the synthesis and binding affinities of these racemized products will be discussed.

##### 4.3.1.1. Synthetic route

As described in **paper 4**, the quinolizine derivatives starting respectively from methyl D- and L-tryptophanate were prepared. These quinolizine were converted to the corresponding quaternary salts **16** and **17**, which were subsequently hydrolyzed in an alkaline medium at 0 °C to afford the carboxylic acid derivatives **18** and **19**, as described in **paper 4**. Subsequent reduction under Birch conditions yielded the amino acid derivatives **20** and **21**. (**Paper 4**) These were reduced into the hydroxymethyl derivatives **22** and **23** (**paper 4**), which were in turn acylated by benzoyl and acetyl chloride. In addition, the amino acid derivatives **20** and **21** were converted to the diethylamide **26** and **27** and successively into the diethylaminomethyl derivatives **28** and **29**. (**Figure 4.6**)

However, our investigations (optical rotation and chiral HPLC analysis in co-operation with Dr. Hefnawy, King Saud University) indicated that the precursory azecines **20**, **21**, **22** and **23** were not enantiomerically pure (**paper 4**), and conclusively all the subsequently obtained target azecines.

Racemization evidently took place at a step prior to the hydroxymethyl derivatives **22** and **23**. Reduction of the carboxylic to the hydroxymethyl group using lithium aluminum hydride clearly does not result in racemization; the same reaction conditions were applied elsewhere (**paper 4**), and the enantiopurity of the compounds was maintained.



**Reagents and conditions:** (a) 1N NaOH, MeOH, 0 °C, 2 h; (b) Na<sup>+</sup>, liq. NH<sub>3</sub>, -40 °C, 10 min; (c) LiAlH<sub>4</sub>, dry THF, reflux, 5 h; (d) RCOCl, pyridine, 0 °C - rt, 3h; (e) HN(Et)<sub>2</sub>, EDCxHCl, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, DMF, 40 °C, 24 h.

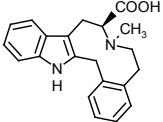
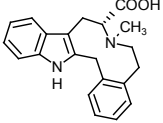
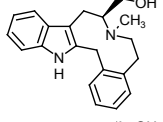
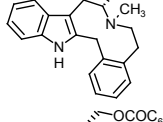
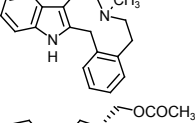
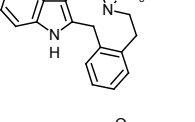
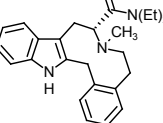
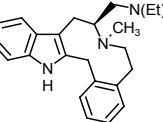
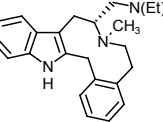
**Figure 4.6:** Scheme for the synthesis of various azecines derived from D- and L-tryptophan.

Hence, finding the “culprit”, i.e. the reaction that resulted in racemization, is not difficult. Into consideration comes primarily the step where the ester function of the quaternary salts **16** and **17** was hydrolyzed to the corresponding carboxylic acids **18** and **19**. Amino acids are generally known to be labile to racemization in alkaline medium. Although the conditions applied for the hydrolysis of **16** and **17** were mild (reaction was carried out at 0 °C), and similar conditions have been conventionally used for the hydrolysis of optically active methyl ester derivatives<sup>114;115</sup>, the quaternary amino acid salts (**18** and **19**) seem to be even more labile to racemization in alkaline medium than conventional amino acid derivatives. The most reasonable explanation is that the α-hydrogen in the quaternary salts **16** and **17** as well as **18** and **19** is more acidic due to the additional neighboring positively charged ammonium function.

#### 4.3.1.2. Affinities for the dopamine receptors

The affinities of the obtained compounds for the dopamine receptors were assessed in a radioligand binding assay. (**Table 4.2**) In view of the fact that the obtained compounds are not enantiopure and that analogous enantiopure compounds showed a great difference in the affinities of the enantiomers (cf. **paper 4**), little can be deduced from the herein measured affinities.

**Table 4.2:** Affinities ( $K_i$ , nM) of racemized 8-substituted LE300 derivatives.

Compounds	$K_i$ (nM), (Radioligand binding experiments)				
	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
<b>20*</b> 	>10000	>10000	>10000	>10000	657 ± 163
<b>21*</b> 	>10000	>10000	n.d.	>10000	1020 ± 368
<b>22*</b> 	44.8 ± 29.6	200.7 ± 89.4	>10000	512 ± 274	93.2
<b>23*</b> 	31.8 ± 13	566 ± 266	n.d.	n.d.	23.1 ± 3.1
<b>24</b> 	17.2 ± 6	430.5 ± 219.5	2214 ± 540	32.8 ± 11.5	16.6 ± 9.2
<b>25</b> 	26.7 ± 2.5	244 ± 27	n.d.	n.d.	12.0 ± 3.8
<b>27</b> 	692.8 ± 279	2300 ± 1158	n.d.	>10000	489.3 ± 35.8
<b>28</b> 	12.8	946 ± 2	n.d.	n.d.	11.4 ± 1.9
<b>29</b> 	29.05 ± 4.9	648.3 ± 145.4	201.00	n.d.	20.9 ± 6.0

\* Paper 4

Indeed, the obtained  $K_i$  values may serve as additional evidence that partial racemization occurred, since no significant difference in the affinities of the ‘supposed’ *R*- and *S*-enantiomers could be observed. (Cf. compounds **22** and **23** as well as **28** and **29**). Determination of the full affinity profile of the derivatives was hence not further pursued.

Nevertheless, some deductions can be made from the determined affinity results when bearing in mind that these target compounds were obtained in an almost completely racemized form. (**Table 4.2**) No significant difference was observed between the racemized hydroxymethyl azecines **22** and **23** and the acylated ones **28** and **29**, indicating that introduction of an acetyl group or even a bulkier benzoyl group might still be well tolerated at the receptor binding site. A diethylaminomethyl residue (compounds **28** and **29**) seems to be similarly well tolerated, whereas its amide counterpart (compounds **26** and **27**) showed a pronounced decrease in affinities. The most significant decrease in affinities was observed with the carboxylic acid residue (compounds **20** and **21**). This was however highly remarkable, since they lacked affinities for all dopamine receptors, except for  $D_5$ . (**Paper 4**)

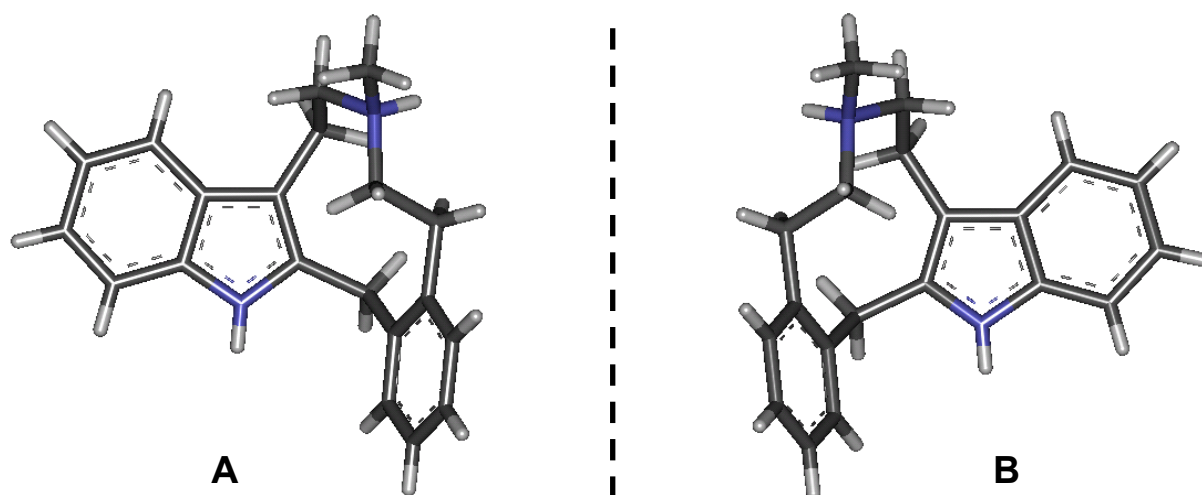
#### 4.3.2. Enantiopure 8*R*- and 8*S*-methyl benzindoloazecine: Understanding the reason behind the discrepancy in their affinities

As mentioned in **paper 4** the *S*-enantiomer of the 8-methylated LE300 showed almost no difference in affinities to the lead LE300 while the *R*-enantiomer was at least 100-fold less active, showing micromolar affinities for the  $D_1$ -like receptors and practically no affinities for  $D_2$ -like receptors ( $K_i > 10000$  nM). The vast difference in the affinities of the *R*- and *S*-enantiomers could only be attributed to their different spatial arrangement, which in turn affects their binding to the receptor binding pocket. In order to picture these derivatives in space (no crystal structure could be obtained for the separated enantiomers), the X-ray structure (CCDC-198889) of the parent compound LE300 served as starting point.

As depicted in **figure 4.7**, LE300 HCl has a folded structure (cage-like structure), where the central azecine ring is present in the form of a cage with both aromatic moieties at each side and the *N*-methyl group pointing into this “cage-like” structure. Interestingly, two energetically equivalent structures, that are mirror images to each others, were found in the X-ray structure. If the perspective is fixed so that the structure is bent forward, it becomes obvious that in one structure the indole ring is on the left side (**A**) and in the other on the right (**B**).

This could be perceived as a kind of axial isomerism, originating at the methylene bridge connecting both aromatic rings.



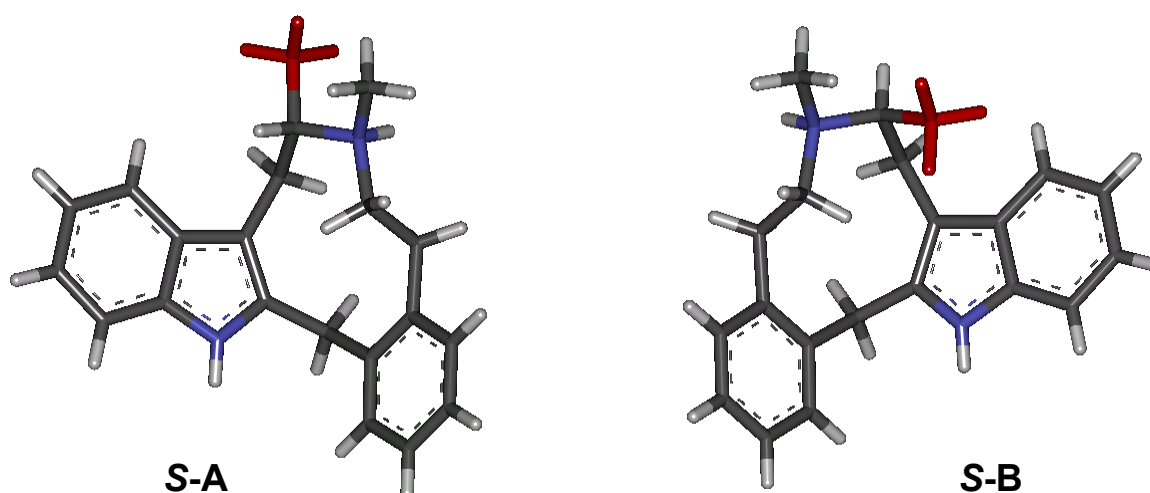


**Figure 4.7:** X-ray structure of LE300 HCl; two ‘enantiomers’ (mirror images; energetically equivalent) are found in a 1:1 ratio.

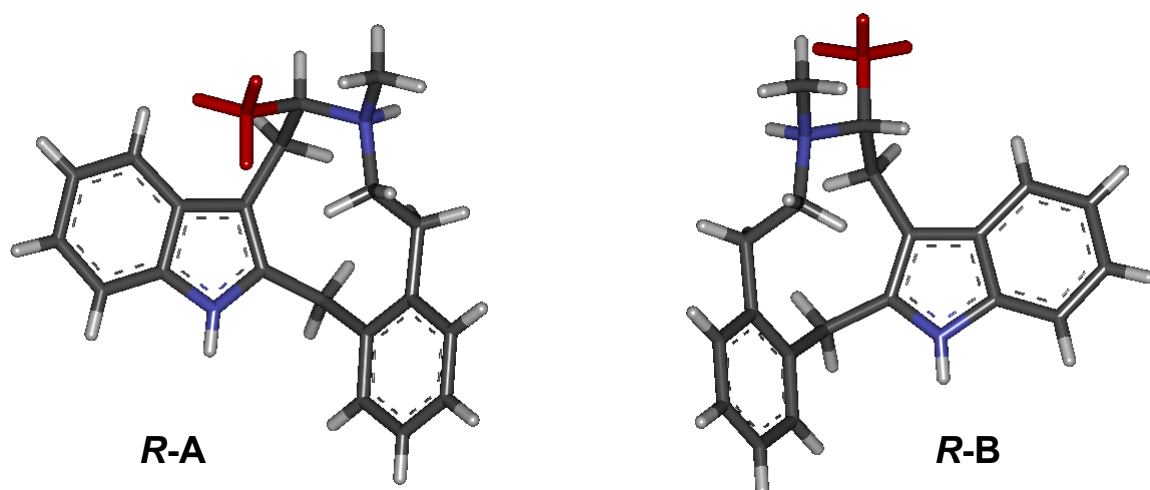
To visualize the spatial arrangement of the obtained (8*S*)- and (8*R*)-methylated indolobenzazecines, one of the H-atoms in each of the aforementioned “mirror images” of LE300 HCl (**figure 4.7, A and B**) was replaced by a methyl group, so that it bears an *S*- or an *R*-configuration. (**Figure 4.8: S-A and S-B**; and **figure 4.9: R-A and R-B**, respectively).

The following observations can be made from **figures 4.8** and **4.9**:

- Unlike LE300 (**figure 4.7**), the structures of both possible axial isomers obtained for the *S*-enantiomer (**figure 4.8**) are not mirror images and thus energetically nonequivalent. The same applies for the *R*-enantiomer.
- The position of the additional methyl group (in red) is highly different in both axial isomers (compare **S-A** and **S-B**). In one structure the methyl group points into the bend azecine ring (**S-B**) and in the other outwards (**S-A**).
- **S-A** and **R-B** are mirror images to each other and energetically equivalent, the same goes for **S-B** and **R-A**.



**Figure 4.8:** Insertion of an (8*S*)-methyl group (in red) at position 8 of both 'enantiomers' found in the X-ray of LE300 HCl.



**Figure 4.9:** Insertion of an (8*R*)-methyl group (in red) at position 8 of both 'enantiomers' found in the X-ray of LE300 HCl.


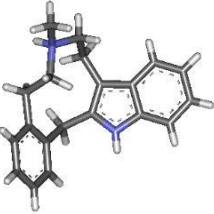

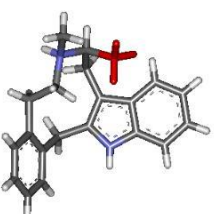
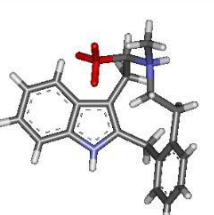
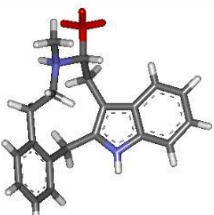
In conclusion, either **S-A** and **R-B** or **S-B** and **R-A** could exist as the possible conformers of the *S*- and *R*-enantiomers, respectively. This depends on the position of the 8-methyl group (pointing inwards or outwards the cage-like structure) which is energetically more favored. This means that the additional methyl group at position 8 would most probably force the ring into only one of the axial isomeric forms unlike LE300.

- Consequently, a major difference between the *S*- and *R*-enantiomer would lie in the position of the indole and benzene ring with respect to the curved central azecine structure, in addition to the position of the 8-methyl group (left or right).

- This implies that the reason behind the activity of the *S*-enantiomer and the inactivity of the *R*-enantiomer might result from the spatial orientation of the axial isomer (indole to the left or to the right of the bent structure).
- Unraveling the energetically more favored structure will not only help decipher the reason behind the high discrepancy in the affinity of the *S*- and *R*-enantiomer, but also reveal which of the axial isomers of LE300 is more active at the receptor binding site.

As a preliminary step, the potential energy of the four different forms (**S-A**, **S-B**, **R-A** and **R-B**) in addition to both forms **A** and **B** of LE300 HCl was calculated. For this purpose, the Dreiding energy (as a parameter of the potential energy) was calculated using Chemaxon 5.3.0. The results obtained are given in **table 4.3**.

**Table 4.3:** Calculated potential energy of the different conformations.

Structure	Energy (Kcal/mol)	Structure	Energy (Kcal/mol)
<b>A</b> (LE300 HCl) 	202.97	<b>B</b> (LE300 HCl) 	202.97
<b>S-A</b> 	217.16	<b>S-B</b> 	527.45
<b>R-A</b> 	527.45	<b>R-B</b> 	217.16

Based on the calculated energy results, the conformations where the 8-methyl group points outwards, i.e. **S-A** and **R-B**, are energetically markedly more favored than those where the 8-methyl group points into the cage-like structure (**S-B** and **R-A**). Nevertheless, the determination of the lowest energy conformer by crystal structure or molecular dynamics is still needed to ascertain these findings.

Assuming that the *S*- and *R*- enantiomer exist in the **S-A** and **R-B** conformation, respectively, two reasons might lie behind the discrepancy in their affinities. First, the position of the indole ring with respect to the bend structure (left in *S*-, right in the *R*-enantiomer). This might indicate that the indole ring to the left side is more favored at the receptor binding pocket; which would also be applicable for unsubstituted benzindoloazecines such as the lead compound LE300.

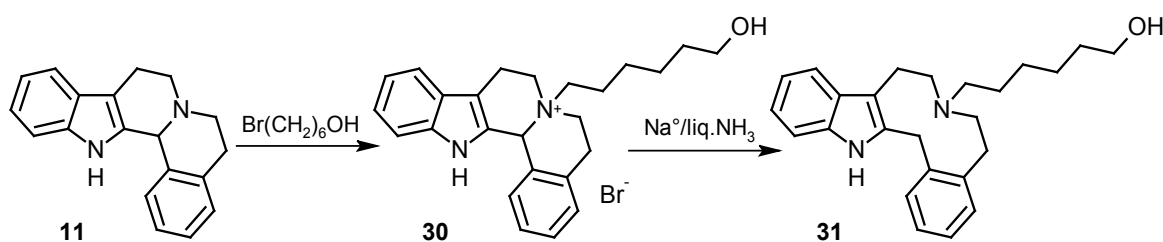
Second, the position of the 8-methyl group might also be of influence; to the left there could be enough space (*S*-enantiomer) while to the right it might present a steric hindrance at the binding pocket (*R*-enantiomer).

More calculations and modelling approaches will be necessary to confirm these assumptions.

#### 4.4. Substitution of the alicyclic *N* with longer and functionalized side chains

##### 4.4.1. Synthesis of *N*-hydroxyhexyl LE300

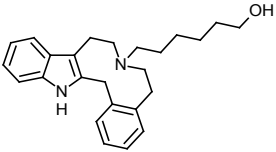
Previous studies have shown that a methyl substituent at the central alicyclic nitrogen is most favored, while longer substituents resulted in decreased affinities. Nevertheless, it remains of interest to examine how longer substituents bearing a functional group would affect the activities towards the dopamine receptors and whether a selectivity towards one of the receptor subtypes could be achieved. For this purpose, the *N*7-(6-hydroxyhexyl)benzindoloazecine derivative **31** was prepared, and its affinities for the dopamine receptor subtypes were tested.



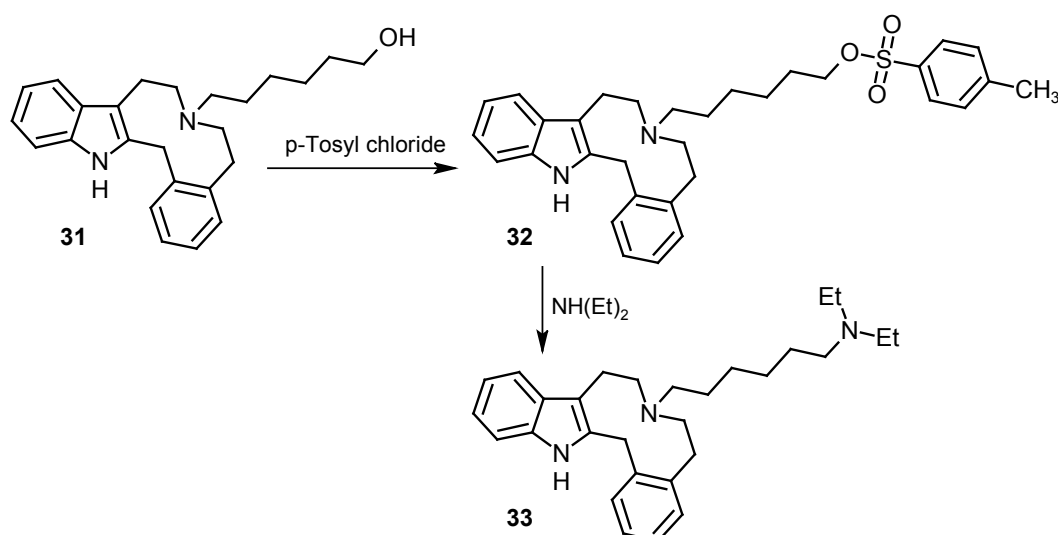
**Figure 4.10:** Synthesis of an LE300 derivative bearing a hydroxyhexyl chain at the alicyclic nitrogen.

The target azecine was prepared in two steps. First the quinolizine **11** was treated with two-molar excess of 6-bromohexanol - only very poor results were achieved with equimolar 6-bromohexanol - and the resulting quaternary salt **30** was then reduced under Birch conditions to afford the target azecine derivative **31**. (**Figure 4.10**)

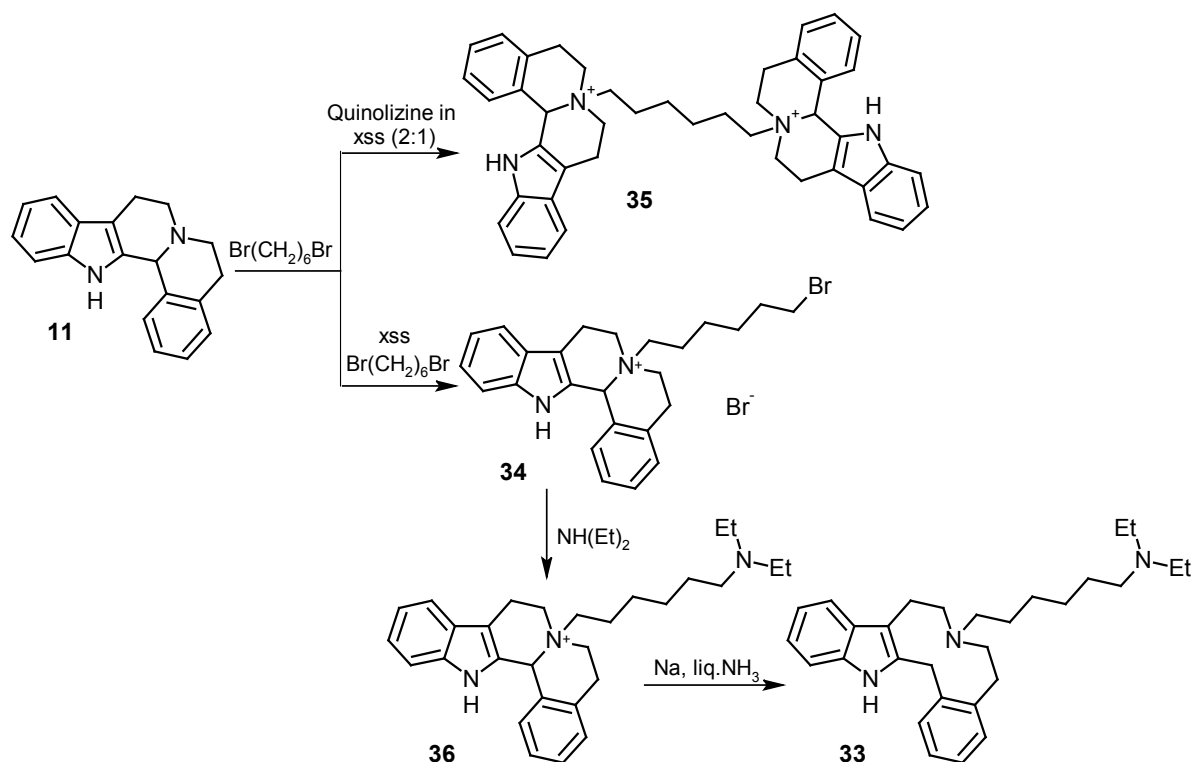
**Table 4.4:** Affinities ( $K_i$ ; nM) of *N*7-(6-hydroxyhexyl)benzindoloazecine derivative **31**.

Compound	$K_i$ (nM), (Radioligand binding experiments)		
	D <sub>1</sub>	D <sub>2</sub>	D <sub>5</sub>
<b>31</b> 	208 ± 28	315	70.1

Trials to further derivatize the hydroxyl group into a diethylamine function through the tosylated derivative **32** failed. (**Figure 4.11**) The product obtained from the tosylation reaction was highly impure and could not be purified, especially because of its low yield.

**Figure 4.11:** Trial to convert the hydroxyl to a diethylamine function.

Another possible way of obtaining the *N,N*-diethylated derivative **33** is through selective quaternization of the quinolizine by dibromoalkane to the bromoalkyl quinolizinium salt **34**. For this purpose the dibromoalkane should be used in excess to avoid the formation of the bivalent derivative **35**. The formed quaternary salt **34** could then be derivatized by diethylamine then reduced under Birch conditions to obtain the desired azecine derivative. (**Figure 4.12**) Advantages of this method would be that different dibromoalkanes with varying chain length (which are easier to obtain and relatively inexpensive compared to bromoalcohol derivatives) could be used and derivatization of the obtained quaternary salts could be achieved by various amines. This would secure a wide variety of products enabling a more thorough SAR study.



**Figure 4.12:** Alternative route for the synthesis of LE300 derivatives with long functionalized chains at the alicyclic nitrogen.

First trials to follow this route were promising. The bromohexyl quaternary salt **34** was obtained in a relatively pure form;  $^1\text{H}$ -NMR showed mainly the target quaternary salt **34** besides traces of the bivalent derivative **35**.

#### 4.5. Synthetic procedures, spectral and analytical data

**4,5,11b,13-Tetrahydro-7H-isoindolo[2',1':1,2]azepino[3,4,5-cd]indol-7-one (5):** To a solution of 4-aminoethylindole (3.38 g, 24 mmol) and 2-formylbenzoic acid (3.59 g, 24 mmol) in a mixture of 120 ml ethanol and 120 ml water was added 24 ml 2N H<sub>2</sub>SO<sub>4</sub> and the reaction mixture was heated under reflux for 48 h. The product separated out as a black solid which was filtered off, washed with a mixture of water and ethanol (1:1) and dried. Yield 71%; m.p. 226-228 °C. <sup>1</sup>H-NMR: 250 MHz (MeOD-*d*<sub>4</sub>): δ 3.30-3.59 (m, 2H, aliphatic), 3.82-3.93 (m, 1H, aliphatic), 3.20-3.30 (m, 1H, aliphatic), 6.20 (s, 1H, indole-CH-benzene), 6.82-6.84 (d, *J*=7, 1H, aromatic), 6.99-7.02 (t, *J*=7, 1H, aromatic), 7.12-7.22 (d, *J*=8, 1H, aromatic), 7.31 (s, 1H, aromatic), 7.51-7.54 (t, *J*=7, 1H, aromatic), 7.66-7.79 (m, 2H, aromatic), 7.92-7.95 (d, *J*=8, 1H, aromatic).

**2,12b-Dihydroisoindolo[2,1-*b*]pyrrolo[4,3,2-*de*]isoquinolin-8(6*H*)-one (6):** Starting from 4-aminomethylindole (2.34 g, 16 mmol) and 2-formylbenzoic acid (2.4 g, 16 mmol) in a mixture of 80 ml ethanol, 80 ml water and 16 ml 2N H<sub>2</sub>SO<sub>4</sub> the same procedure applied for the synthesis of compound **5** was adopted. A yellowish white solid was obtained. Yield 66%; m.p. 214 °C. <sup>1</sup>H-NMR: 250 MHz (DMSO-*d*<sub>6</sub>): δ 4.55-4.61 (d, *J*=16, 1H, 6), 5.47-5.54 (d, *J*=16, 1H, 6), 6.07 (s, 1H, indole-CH-benzene), 6.93-6.96 (d, *J*=7, 1H, aromatic), 7.06-7.12 (t, *J*=7, 1H, aromatic), 7.20-7.23 (d, *J*=8, 1H, aromatic), 7.46-7.55 (m, 2H, aromatic), 7.67-7.73 (m, 2H, aromatic), 8.08-8.11 (d, *J*=8, 1H, aromatic), 11.05 (s, 1H, indole-NH).

**14b-Ethyl-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinoline (13):** A solution of quinolizine **11** (274 mg, 1 mmol) and iodine (1.02 g, 4 mmol) in 70 ml ethanol was refluxed under nitrogen for 3.5 h. The solvent was removed under vacuum and then a saturated solution of sodium thiosulfate was added until the color of the mixture faded to yellow. The product was extracted with chloroform, the solvent removed under reduced pressure, leaving the imminium salt as a yellow solid.

To a suspension of Mg-turnings (100 mmol) in dry diethyl ether was added ethyl iodide (5 mmol) and the suspension was warmed to 45 °C for 30 min. The reaction mixture was subsequently cooled in an ice-bath and a solution of the obtained imminium salt **12** (400 mg, 1 mmol) in dry THF was slowly added. The ice bath was removed and the mixture refluxed for 5 h under nitrogen. The reaction was quenched by dropwise addition of water, and the product was extracted with diethyl ether. The organic solvent was dried over MgSO<sub>4</sub> evaporated under reduced pressure leaving a creamy white solid, which was column chromatographed using dichloromethane/methanol (75:2) as eluent. Yield 66%; m.p. 109 °C. <sup>1</sup>H-NMR 250 MHz (CDCl<sub>3</sub>): δ 0.73-0.79 (t, *J*=7, 3H, CH<sub>3</sub>CH<sub>2</sub>), 2.26-2.34 (q, *J*=7, 2H, CH<sub>3</sub>CH<sub>2</sub>), 2.67-2.91, 2.94-3.21, 3.48-3.64 (mc, 8H, 5, 6, 8, 9), 7.05-7.35 (m, 6H, aromatic), 7.48-7.50 (d, *J*=7, 1H, aromatic), 7.57-7.60 (d, *J*=8, 1H, aromatic), 7.82 (s, 1H, indole-NH).

Anal. calcd. for  $C_{21}H_{22}N_2 \times 0.4 CH_2Cl_2$ : C 79.72%, H 7.07%, N 8.77%; found C 79.80%, H 7.05%, N 8.55%.

**15-Ethyl-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine (10):** The quinolizine **13** was quaternized with methyl iodide and the obtained salt was reduced under Birch conditions following previously described procedures.<sup>105</sup> White waxy solid. Yield 66%; m.p. 106 °C. <sup>1</sup>H-NMR 250 MHz (CDCl<sub>3</sub>):  $\delta$  0.92-0.98 (t,  $J=7$ , 3H, CH<sub>3</sub>-CH<sub>2</sub>), 2.04-2.21 (m, 2H, CH<sub>3</sub>CH<sub>2</sub>), 3.22 (s, 3H, N-CH<sub>3</sub>), 2.52-3.54 (m, 8H, 5, 6, 8, 9), 4.85-4.91 (t,  $J=8$ , 1H, 15), 7.05-7.28 (m, 6H, aromatic), 7.43-7.46 (m,  $J=7$ , 2H, aromatic), 7.88 (s, 1H, indole-NH). Anal. calcd. for  $C_{22}H_{26}N_2 \times H_2O$ : C 78.53%, H 8.39%, N 8.33%; found C 78.76%, H 8.68%, N 8.11%.

**15-Methoxy-5,6,8,9,14,15-hexahydro-7H-indolo[3,2-f][3]benzazecine-7-carbonitrile (14):** To a solution of the quinolizine **11** (356 mg, 1.3 mmol) in dry THF and dry methanol (4:1) was added magnesium oxide (312 mg, 7.2 mmol) and cyanogen bromide (234 mg, 2.21 mmol). The reaction mixture was stirred at room temperature for 2 h, filtered off to get rid of magnesium oxide, and the filtrate was removed under reduced pressure. The residue was suspended in water and the product extracted with dichloromethane. The organic layer was dried over MgSO<sub>4</sub> and evaporated under reduced pressure leaving a white solid. Yield 93%; m.p. 213-214 °C <sup>1</sup>H-NMR: 250 MHz (CDCl<sub>3</sub>):  $\delta$  2.26-2.66, 2.89-3.01, 3.05-3.08, 3.18-3.32 and 3.88-3.90 (m, 8H, 5, 6, 8 and 9), 3.37 (s, 3H, OCH<sub>3</sub>), 5.96 (s, 1H, 15), 7.05-7.11 (t,  $J=7$ , 1H, aromatic), 7.16-7.26 (m, 2H, aromatic), 7.32-7.42 (m, 4H, aromatic), 7.51-7.54 (d,  $J=7$ , 1H, aromatic), 8.92 (s, 1H, indole NH). <sup>13</sup>C-NMR: 250 MHz (CDCl<sub>3</sub>):  $\delta$  23.76 (5), 32.86 (9), 55.40 (8), 56.07 (OCH<sub>3</sub>), 56.28 (6), 74.75 (15), 107.85 (9a), 111.21 (13a), 117.45 (11), 118.01 (CN), 119.24 (10), 121.99 (12), 128.02 (3), 128.06 (9b), 128.18 (2), 128.92 (1), 129.55 (4), 134.77 (14a), 136.95 (4a), 138.41 (13a), 138.56 (15a).

**(7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecin-8-yl)methyl benzoate (24)** To an ice-cold solution of the methylalcohol derivative **23** (150 mg, 0.47 mmol) in 2 ml pyridine was added benzoyl chloride (70 mg, 0.5 mmol), and the reaction mixture was stirred at room temperature for three hours. It was then poured onto ice water and extracted with dichloromethane. The organic layer was washed with 0.1 N HCl, with water and then with a dilute solution of sodium bicarbonate, and finally evaporated under reduced pressure. The obtained product was purified by column chromatography using ethyl acetate/hexane (1/1). Yield 53.1%; m.p. 70-71 °C. <sup>1</sup>H-NMR: 400 MHz (CDCl<sub>3</sub>):  $\delta$  2.41-2.46 (m, 1H, aliphatic), 2.46 (s, 3H, N-CH<sub>3</sub>), 2.64-2.69 (m, 1H, aliphatic), 2.83-2.90 (m, 3H, aliphatic), 2.99-3.04 (m, 1H, aliphatic), 3.12-3.18 (m, 1H, aliphatic), 4.25-4.28 (d,  $J=15$ , 1H, 15), 4.37-4.54 (m, 3H, 15 and CH<sub>2</sub>OH), 7.06-7.10 (t,  $J=7$ , 1H, aromatic), 7.13-7.22 (m, 4H, aromatic), 7.28-7.32 (m, 2H, aromatic), 7.46-7.53 (m, 3H, aromatic), 7.57-7.64 (m, 1H, aromatic), 7.98 (s, 1H, indole-NH),



8.09-8.14 (m, 2H, aromatic). Anal. calcd. for  $C_{28}H_{28}N_2O_2 \times \frac{1}{3}EtOAc$ : C 77.62%, H 6.81%, N 6.17%; found C 77.34%, H 6.59%, N 5.80%.

**(7-Methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecin-8-yl)methyl acetate (25)** The methylalcohol derivative **23** (128 mg, 0.4 mmol) was acetylated using acetyl chloride (35 mg, 0.44 mmol), following the same acylation procedure described for compound **24**. The product was chromatographed with dichloromethane/MeOH (19:1). Yield 35%; m.p. 69-70 °C.  $^1H$ -NMR: 250 MHz ( $CDCl_3$ ):  $\delta$  2.13 (s, 3H,  $COCH_3$ ), 2.32-2.42 (m, 1H, aliphatic), 2.38 (s, 3H, N- $CH_3$ ), 2.58-3.01 (m, 6H, aliphatic), 4.04-4.30 (m, 3H, H-15 and  $CH_2OH$ ), 4.36-4.42 (d,  $J=15$ , 1H, 15), 7.06-7.30 (m, 7H, aromatic), 7.46-7.48 (d,  $J=7$ , 1H, aromatic), 7.96 (s, 1H, indole- $NH$ ). Anal. calcd. for  $C_{23}H_{26}N_2O_2 \times 0.5 MeOH$ : C 74.57%, H 7.46%, N 7.40%; found C 74.64%, H 7.44%, N 7.19%.

***N,N*-Diethyl-7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecine-8-carboxamide (26 and 27):** To an ice-cold suspension of the respective amino acid derivative **20** or **21** (1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride (1.1 mmol) and dimethylaminopyridine (DMAP) (1 mmol) in dichloromethane and dimethylformamide (8:1) was added diethylamine (1.05 mmol). The reaction mixture was stirred at 40°C for 24 h, and subsequently washed with a diluted solution of sodium bicarbonate then with water. The organic layer was evaporated under reduced pressure and the obtained brownish oil was purified by column chromatography using a mixture of dichloromethane/MeOH (25/1) as eluent giving a creamy white solid. Yield 32-40%; m.p. 104-106 °C.  $^1H$ -NMR: 400 MHz ( $CDCl_3$ ):  $\delta$  1.15-1.19 (t,  $J=7$ , 3H,  $CH_3CH_2N$ ), 1.19-1.23 (t,  $J=7$ , 3H,  $CH_3CH_2N$ ), 2.14 (s, 3H,  $CH_3$ ), 2.68-2.83 (m, 2H, aliphatic), 2.89-2.97 (m, 2H, aliphatic), 3.06-3.13 (m, 1H, aliphatic), 3.32-3.65 (m, 5H, aliphatic), 3.80-3.83 (dd,  $J=10$ , 2, 1H, 8), 4.18-4.22 (d,  $J=15$ , 1H, 15), 4.37-4.41 (d,  $J=15$ , 1H, 15), 7.07-7.19 (m, 5H, aromatic), 7.28-7.31 (m, 2H, aromatic), 7.47-7.49 (d,  $J=7$ , 1H, aromatic), 8.15 (s, 1H, indole- $NH$ ).  $^{13}C$ -NMR: 400 MHz ( $CDCl_3$ ):  $\delta$  13.26 ( $CH_3CH_2N$ ), 14.71 ( $CH_3CH_2N$ ), 26.41 (5), 31.85 (9), 34.65 (15), 40.17 ( $CH_3CH_2N$ ), 41.75 ( $CH_3CH_2N$ ), 42.18 (N- $CH_3$ ), 53.14 (6), 64.46 (8), 110.43 (9a), 110.50 (13), 117.91 (10), 119.19 (11), 121.21 (12), 126.39 (3), 126.85 (1), 128.76 (9b), 130.22 (2), 130.74 (4), 134.92 (14a), 135.34 (13a), 137.21 (4a), 140.16 (15a), 172.38 (C=O). Anal. calcd. for  $C_{25}H_{31}N_3O \times 0.25 H_2O$ : C 76.20%, H 8.06%, N 10.66%; found C 76.23%, H 8.11%, N 10.44%.

***N,N*-Diethyl-*N*-[(7-methyl-6,7,8,9,14,15-hexahydro-5H-indolo[3,2-f][3]benzazecin-8-yl)-methyl]amine (28 and 29):** The respective amide (**26**, **27**) was reduced using two molar excess of lithium aluminum hydride in tetrahydrofuran. A creamy white solid was obtained after purification by column chromatography (dichloromethane/methanol 20:1). Yield 80-81%; m.p. 75-76 °C.  $^1H$ -NMR: 250 MHz ( $CDCl_3$ ):  $\delta$  1.03-1.09 (t,  $J=7$ , 6H,  $CH_3CH_2N$ ), 2.18-2.31 (m, 2H, aliphatic), 2.36 (s, 3H,  $CH_3$ ), 2.43-3.05 (m, 11H, aliphatic), 4.21-4.27 (d,  $J=15$ , 1H, H-

15), 4.40-4.46 (d,  $J=15$ , 1H, H-15), 7.05-7.30 (m, 7H, aromatic), 7.61-7.65 (d,  $J=8$ , 1H, aromatic), 7.85 (s, 1H, indole-*NH*).  $^{13}\text{C}$ -NMR: 250 MHz ( $\text{CDCl}_3$ ):  $\delta$  12.04 ( $\text{CH}_3\text{CH}_2\text{N}$ ), 26.88 (5), 32.73 (9), 34.54 (15), 41.53 (N- $\text{CH}_3$ ), 47.34 ( $\text{CH}_3\text{CH}_2\text{N}$ ), 53.53 (6), 45.25 ( $\text{CH}_2\text{N}(\text{Et})_2$ ), 63.33 (8), 110.43 (13), 110.63 (9a), 118.23 (10), 119.30 (11), 121.51 (12), 126.69 (3), 127.17 (1), 128.54 (9b), 130.26 (2), 130.69 (4), 133.39 (14a), 135.61(13a), 136.93 (4a), 140.07 (15a). Anal. calcd. for  $\text{C}_{25}\text{H}_{33}\text{N}_3 \times 0.8 \text{ H}_2\text{O}$ : C 77.00%, H 8.94%, N 10.78%; found C 76.69%, H 8.69%, N 10.46%.

**7-(6-Hydroxyhexyl)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinolin-7-ium bromide (30):** A solution of the quinolizine **11** (493 mg, 1.8 mmol) and 6-bromohexanol (391 mg, 3.24 mmol) in toluene was heated under reflux for 24 h. The separated yellow solid was filtered off and dried. Yield 48.9%; m.p. 270-271 °C.  $^1\text{H}$ -NMR: 250 MHz ( $\text{DMSO}-d_6$ ):  $\delta$  1.26-1.38 (m, 6H, aliphatic), 1.91 (mc, 2H, aliphatic), 3.12-3.45 (m, 7H, aliphatic), 3.89-3.98 (m, 4H, aliphatic), 4.32-4.36 (m, 1H, aliphatic), 6.20 (s, 1H, 14b), 7.01-7.07 (t,  $J=7$ , 1H, aromatic), 7.11-7.17 (t,  $J=7$ , 1H, aromatic), 7.36-7.53 (m, 6H, aromatic), 10.82 (s, 1H, indole-*NH*).

**7-(6-Hydroxyhexyl)-6,7,8,9,14,15-hexahydro-5*H*-indolo[3,2-*f*][3]benzazecine (31):** The quaternary salt **30** was reduced under Birch conditions following the conventional procedure. The obtained yellowish oil was converted to the HCl salt and triturated with hexane, leaving a yellowish white solid, which was reconverted to the base and extracted with dichloromethane. Yield 72.6%; m.p. 80-81 °C.  $^1\text{H}$ -NMR: 400 MHz ( $\text{DMSO}-d_6$ ):  $\delta$  1.25-1.34 (m, 4H, aliphatic), 1.39-1.45 (m, 2H, aliphatic), 1.73-1.77 (m, 2H, aliphatic), 3.01-3.67 (m, 12H, aliphatic), 4.03-4.06 (d,  $J=15$ , 1H, 15), 4.30-4.34 (d,  $J=15$ , 1H, 15), 6.95-6.99 (t,  $J=7$ , 1H, aromatic), 7.02-7.06 (t,  $J=7$ , 1H, aromatic), 7.19-7.20 (m, 2H, aromatic), 7.26-7.31 (m, 2H, aromatic), 7.43-7.45 (d,  $J=7$ , 1H, aromatic), 7.72-7.74 (d,  $J=7$ , 1H, aromatic), 10.62 (s, 1H,  $\text{N}^+-\text{H}$ ), 10.85 (s, 1H, indole-*NH*). HRMS: 377.259 (calcd.  $\text{C}_{25}\text{H}_{33}\text{N}_2\text{O}$  377.259)

**7-(6-Bromohexyl)-5,6,8,9,14,14b-hexahydroindolo[2',3':3,4]pyrido[2,1-*a*]isoquinolin-7-ium bromide (34):** To a solution of quinolizine **11** (0.82 g, 3 mmol) in 30 ml acetone was added a solution of 1,6-dibromohexane (5.83 g, 24 mmol) in 20 ml acetone. The solution was concentrated to 10 ml and warmed to 40°C for 24 h. The formed yellow solid was filtered off and dried. Yield 51.6%; m.p. >300 °C.  $^1\text{H}$ -NMR: 250 MHz ( $\text{DMSO}-d_6$ ):  $\delta$  1.05-1.10 (m, 4H, aliphatic), 1.26-1.45 (m, 4H, aliphatic), 1.72-1.92 (m, 4H, aliphatic), 3.31-3.20 (m, 4H, aliphatic), 3.38-3.42 (m, 4H, aliphatic), 6.21 (s, 1H, 14b), 7.01- 7.07 (t,  $J=7$ , 1H, aromatic), 7.11-7.17 (t,  $J=7$ , 1H, aromatic), 7.39-7.59 (m, 6H, aromatic), 10.83 (s, 1H, indole-*NH*).

## 5. Discussion

With the discovery of the benzindoloazecine derivative LE300 and its dibenzazecine counterpart LE404 a novel class of potent dopamine receptor antagonists has been established, which is characterized by high affinities as well as a general tendency for D<sub>1</sub> selectivity<sup>105;106;109;116</sup>. Modifications of the structure of both lead compounds by changing either the backbone scaffold or the nature or pattern of substitution has usually been accompanied by altered affinities and/or selectivity profile<sup>106-109</sup>. Taking together previous findings with respect to SAR studies within this class of dopamine ligands, it can be deduced that some structural features, which are shared by most highly affine derivatives, are crucial for optimum activity.

- An optimal backbone scaffold is made up of a two aromatic ring system surrounding a central aza-alicyclic (preferably a 10-membered ring). Both aromatic rings should be separated by a methylene group. Moreover a certain degree of conformational flexibility is required; rigid structures like the precursory pentacyclic quinolizines are devoid of activity.
- Regarding the nature of the substituent, an *N*-methyl substitution of the central alicyclic of both benzindoloazecines and dibenzazecines was most favored, longer chains or no substituents both led to decreased affinities<sup>106;117</sup>. Within the dibenzazecine series, a 3-hydroxyl substitution was found to be an essential feature for high affinities<sup>109</sup>.

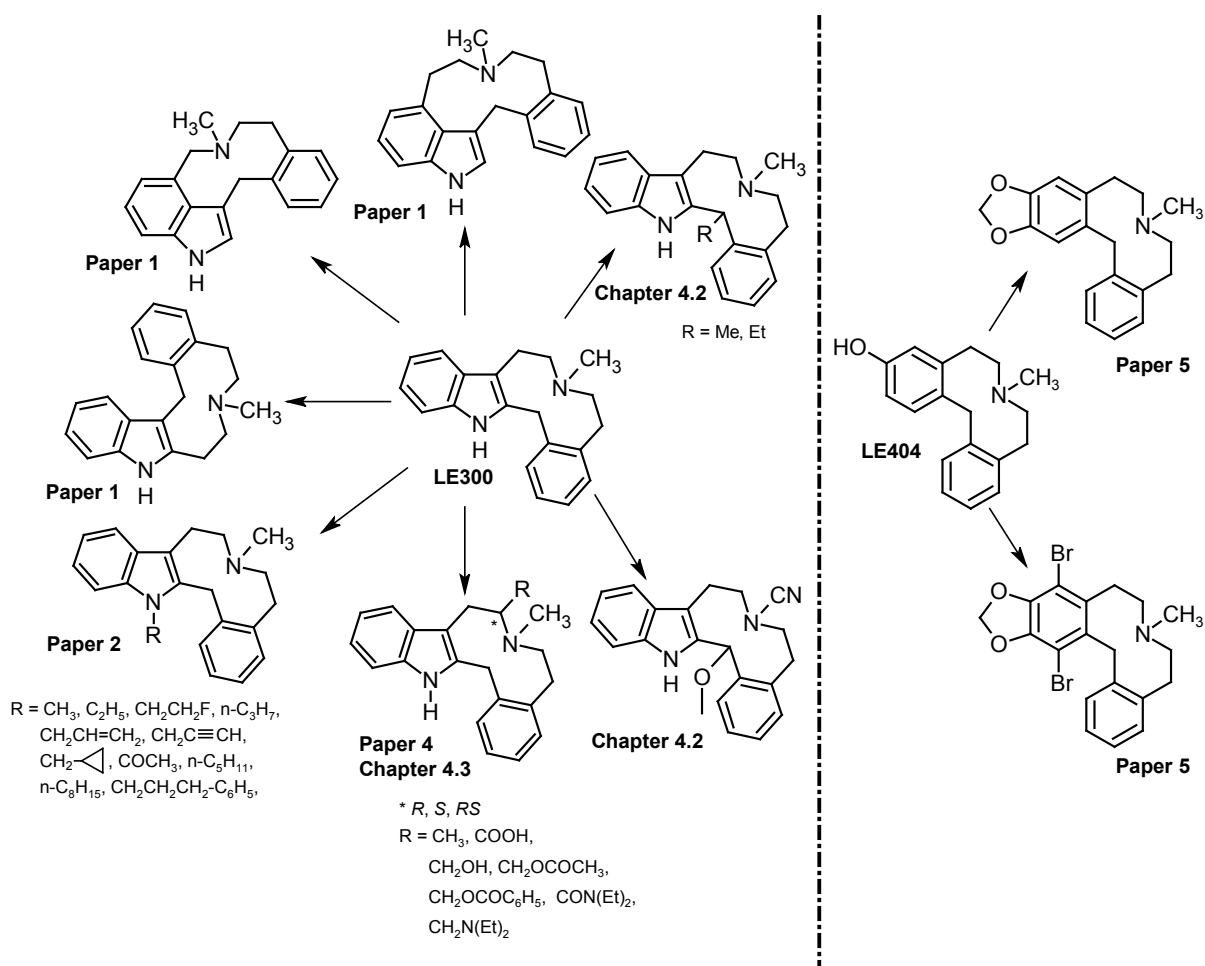
In the present work, these main established structural features were mostly retained and the structures of LE300 and LE404 were otherwise modified.

Modification of LE300 has been accomplished by either changing the annulation pattern of the backbone skeleton (**paper 1**) or by introducing different substituents at various positions, including position 14 (indole-*N*) (**paper 2**), position 15 (the methylene group separating both aromatic rings) (**chapter 4.2**) and position 8 (central ring) (**paper 4** and **chapter 4.3**). LE404 has been modified by replacing the 3-hydroxyl substituent by a methylenedioxy moiety to connect positions 2 and 3. (**paper 5**).

Goal behind these modifications was to try to optimize the structures of both leads to attain higher affinities and/or enhanced selectivity. Moreover, this would help verify and further develop existing SAR studies in order to get better insights into the structural properties, which are determinant for high affinities as well as subtype selectivity. The various structural modifications accomplished in this work are depicted in **figure 5.1**.

All prepared azecines together with some of the precursory compounds were screened for their affinities for the human cloned dopamine receptor subtypes (D<sub>1</sub>-D<sub>5</sub>). The functionality of the compounds was tested by an intracellular calcium assay, where compounds with agonistic properties are characterized by their ability to increase intracellular Ca<sup>2+</sup> levels, whereas antagonists suppress the Ca<sup>2+</sup> influx induced by a standard agonist. A detailed description of the experimental procedures is given in **paper 1**.

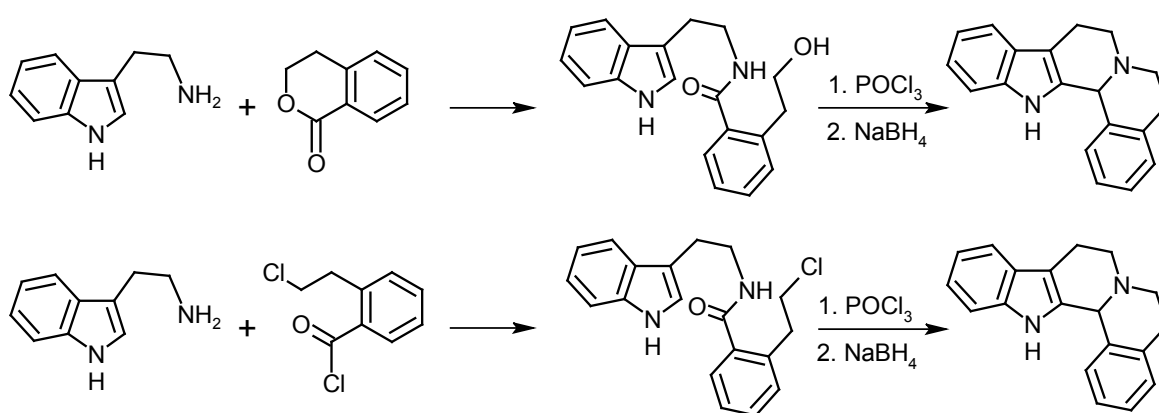
The affinities of all prepared derivatives, together with those of some lead and previously prepared compounds for comparison purposes, are given in **table 5.1**. In the calcium assay; all tested derivatives were found to possess antagonistic activity at the examined dopamine receptors.



**Figure 5.1:** The different structural modifications of both lead compounds LE300 and LE404, which have been accomplished in this work.

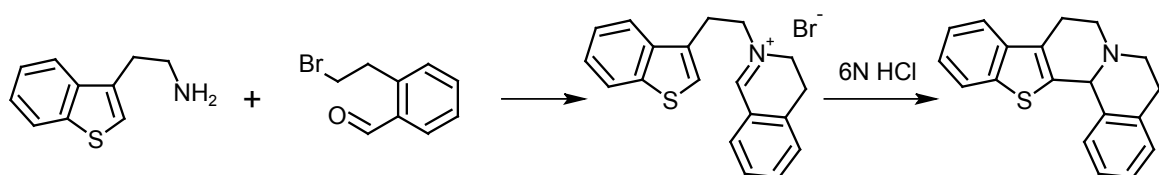
### 5.1. Synthesis of the different quinolizine derivatives as important precursors for the target azecines

Quinolizine derivatives, though themselves usually devoid of dopamine activity, are of value as the sole precursors for the target benzindolo- and dibenzazecines. Hitherto, these quinolizines have been prepared by reacting the respective aralkylamines with either 1-isochromanone<sup>118</sup> or 2-(2-chloroethyl)benzoyl chloride<sup>108</sup>. The resulting hydroxymethylbenzamides were then cyclized under Bischler-Napieralski conditions and the quinolizines were finally obtained after reduction with NaBH<sub>4</sub>. An example of these synthetic procedures is illustrated in **figure 5.2**.



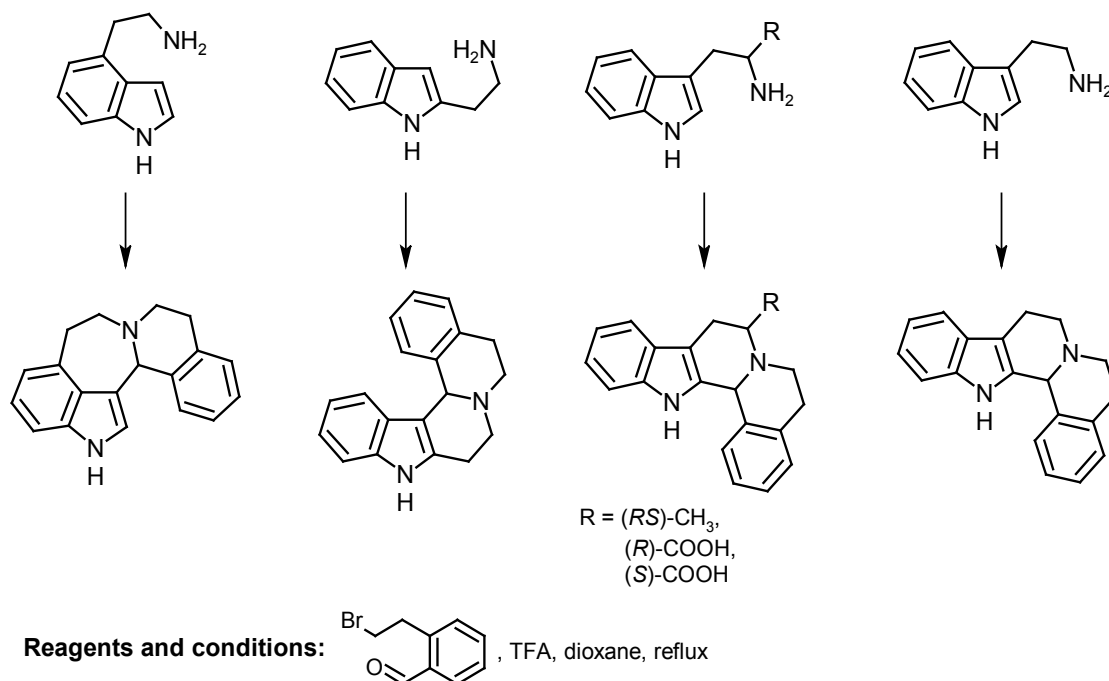
**Figure 5.2:** Previously applied synthetic procedures for the synthesis of quinolizine derivatives.

However, these laborious multistep procedures often resulted in relatively low yields sometimes even failed to produce the desired quinolizines, as is the case with quinolizines starting from 4-aminomethyl- or 4-aminoethylindole. Either no reaction took place, the hydroxyethylbenzamide was formed in very low yields or obtained in a highly impure form. Alternatively, it has been described that analogous quinolizine derivatives (e.g. benzothienoethylamine and thienoethylamine) could be prepared by reacting the aralkylamine derivative with 2-(2-bromoethyl)benzaldehyde followed by cyclization of the formed isoquinolinium salt by a Pictet Spengler reaction using HCl<sup>113</sup>. (**Figure 5.3**)



**Figure 5.3:** Synthesis of a quinolizine derived from benzothienoethylamine as described by Browne<sup>113</sup>.

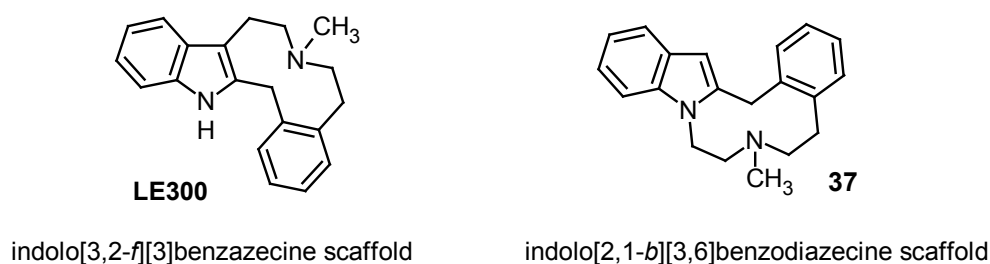
However, some of the aralkylamines, namely tryptamine, indole-2- and -4-ethylamine, failed to produce the intermediary isoquinolinium salt when the described reaction conditions were applied. Hence, the reaction conditions were modified and the amine derivatives were refluxed in the presence of trifluoroacetic acid with 2-(2-bromoethyl)benzaldehyde to directly afford the desired quinolizines. This new established one-pot synthetic procedure for the synthesis of quinolizines could be regarded as an easily applicable route, which in contrast to the previously applied ones, is less laborious and time-consuming and yields the target compounds in relatively good yields. The described procedure has been used to prepare quinolizine derivatives of indol-4-ethylamine, -2-ethylamine (**paper 1**),  $\alpha$ -methyltryptamine, as well as D- and L- tryptophan (**paper 4**). Moreover, the quinolizine precursor of the lead compound LE300 was alternatively prepared using this method (**paper 2**), which is simpler and more efficient than the previously applied one<sup>105;118</sup>. (**Figure 5.4**)



**Figure 5.4:** Different quinolizines prepared starting from the respective indolethylamines and 2-(2-bromoethyl)benzaldehyde.

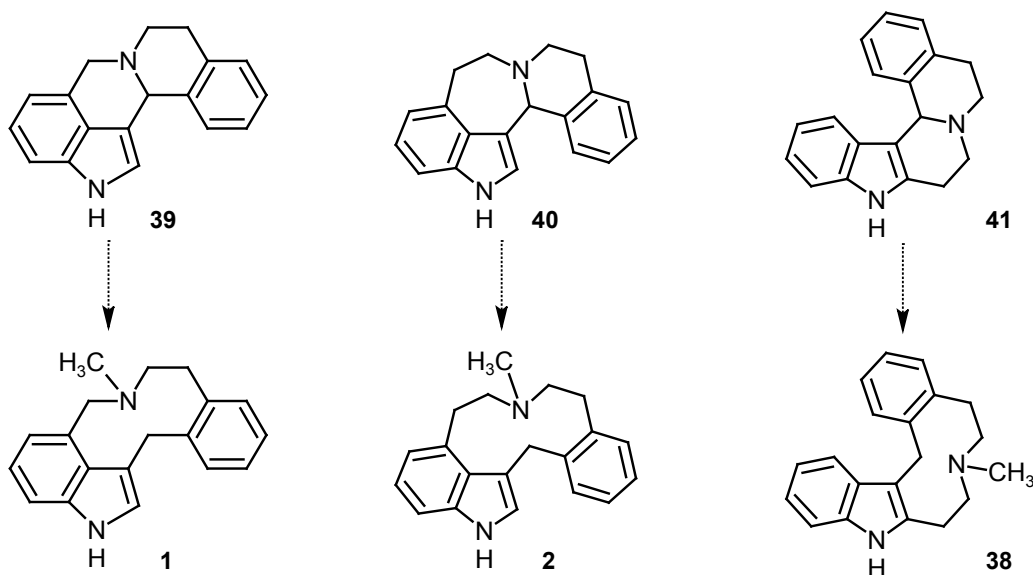
## 5.2. Modulation of the annulation pattern of LE300

Previous investigations have shown that changing the annulation pattern of LE300 from an indolo[3,2-*f*][3]benzazecine to an indolo[2,1-*b*][3,6]benzodiazecine scaffold (compound **37**, **figure 5.5**) results in an inversion of the selectivity profile; the latter derivative displaying 100-fold lower affinity for D<sub>1</sub> than the lead LE300 and subsequently a D<sub>2</sub> over D<sub>1</sub> selectivity<sup>119</sup>.



**Figure 5.5:** LE300 and its analogue **37** with an altered scaffold<sup>119</sup>.

In **paper 1** the annulation pattern of LE300 was further modified in order to assess how the different backbone structures affect the selectivity and affinity for the dopamine receptors. Three LE300 analogues **1**, **2** and **38** and their pentacyclic precursors **39-41** (**figure 5.6**), all of a novel heterocyclic ring structure, were prepared and tested for their affinities for the dopamine receptor subtypes (D<sub>1</sub>-D<sub>5</sub>).



**Figure 5.6:** LE300 analogues with modified structural backbone and their pentacyclic precursors.

Results of the radioligand binding experiments (cf. **table 5.1**) revealed that changing the annulation pattern of LE300 did not lead to pronounced changes, in contrast to what was previously observed with the indolo[2,1-*b*][3,6]benzodiazecine derivative **37**<sup>119</sup>. Analogue **2** showed comparable affinities with LE300, whereas **38** displayed a noticeable decrease in affinities; however they were still in the nanomolar range. Regarding the selectivity profile, a slight change could be observed; the affinities of compounds **2** and **38** for the D<sub>2</sub> with respect to D<sub>1</sub> receptors increased and thus the D<sub>1</sub> selectivity, common to other azecine-type dopamine receptor antagonists, has been lost.

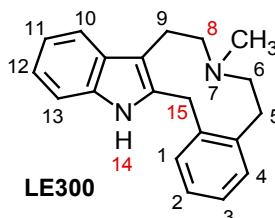
It seems, however, that decreasing the distance between the alicyclic nitrogen and the indole ring with the accompanied decrease in conformational flexibility can result in a complete loss of affinities. This is evident when comparing the affinities of both homologues **1** and **2**. The more rigid analogue **1**, where the alicyclic nitrogen is connected through a methylene group to the indole ring, displayed no significant affinities ( $K_i > 10000$  nM), whereas the ring expanded and hence more flexible analogue **2** showed similar affinities to the lead LE300. This discrepancy in affinities could be explained by the fact that compound **1**, though containing an central azecine moiety like LE300, is much more constrained as a consequence of its annulation pattern. Moreover, the distance between the central nitrogen and the indole ring, both important pharmacophores, is reduced. In contrast, compound **2** with the ring expanded structure shows higher flexibility as well as similar distances between the pharmacophoric groups as in LE300.

In line with our previous findings, the rigid pentacyclic precursor compounds **39-41**, had generally weak to no affinities. The quinolizine **39** displayed no affinities ( $K_i > 10000$  nM) for all dopamine receptors, its homologue **40** showed micromolar affinities for the D<sub>1</sub>, D<sub>2</sub>, and D<sub>5</sub> subtypes and the quinolizine **41** showed micromolar ones for the D<sub>1</sub> and D<sub>2</sub> subtypes. (**Table 5.1**)



### 5.3. Substitution of LE300 at different positions

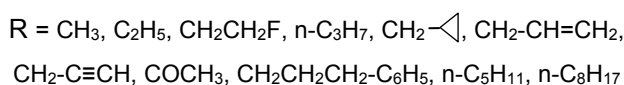
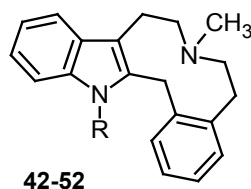
A major part of this work has focused on preparing LE300 derivatives, substituted at different positions, mainly position 14 (indole-*NH*), position 15 (methylene bridge) and position 8. (The positions are marked red in **figure 5.7**)



**Figure 5.7:** Positions of LE300 (in red), which have been modified in this work.

#### 5.3.1. *N*14-substituted LE300 derivatives

A series of eleven LE300 derivatives (**42-52**) (**figure 5.8**) substituted at the indole-*NH* with different residues, ranging from saturated to unsaturated chains of different length, bulk and electronic properties, has been prepared and tested for the affinities at the D<sub>1</sub>-D<sub>5</sub> receptors. (**Paper 2**) Both the affinity and selectivity profile of the derivatives were found to be influenced by the nature of the substituent.



**Figure 5.8:** Indole-*NH* substituted LE300 analogues.

All derivatives bearing an alkyl chain at the indole-*NH* retained more or less the D<sub>1</sub>-selectivity profile; except for the *N*-methylated derivative **42**. Compared with LE300, compound **42** showed a 5-15 fold increase in affinities for all dopamine receptors, except for D<sub>1</sub> where it was equally active as LE300. Increasing the chain length led to a decrease in affinities; the ethyl derivative **43** displayed weaker affinities than the methylated one but was still as active as LE300 itself. The most pronounced decrease was perceived with the octyl derivative **52**, which exhibited one of the lowest affinities among all tested compounds. An interesting finding was that the indole-*N*-pentyl derivative **51**, though generally possessing higher affinities than the octyl derivative **52**, displayed lower ones for the D<sub>5</sub> receptor and hence

showed the highest  $D_1/D_5$  selectivity among the indole-*N* alkylated derivatives. This may be an indication that longer chains are better tolerated at the  $D_1$  than at the  $D_5$  receptors.

Modification of the alkyl chains, did not lead to substantial alterations in the affinities, as could be observed with the derivatives bearing fluoroethyl **44** or cyclopropylmethyl **46** moieties.

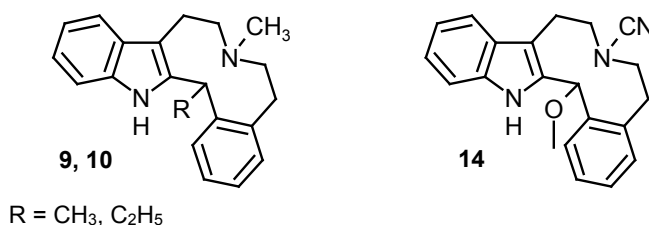
A general increase in the affinities for the  $D_4$  with respect to  $D_1$  receptor could be observed. While LE300 has almost a 60-fold  $D_1$  over  $D_4$  selectivity, all indole-*N* substituted derivatives showed a  $D_1/D_4$  ratio ranging from 0.4- to 20-fold. This was most remarkable with the allylated derivative **47**, which displayed the highest affinity for  $D_4$ , even higher than that for  $D_1$  and equally high to that for  $D_5$ .

### 5.3.2. LE300 bearing substituents between the aromatic rings

Two benzindoloazecine derivatives substituted with methyl<sup>112</sup> or ethyl groups at the methylene moiety (**figure 5.9**), connecting both aromatic rings, were prepared and tested for their affinities. In contrast to indole-*N*-methyl and -ethyl derivatives (**42** and **43**), these compounds (**9** and **10**) displayed a marked decrease in affinities, most pronounced was the decrease in the activity for the  $D_3$  receptor ( $K_i > 10000$  nM). No significant difference in the affinities of the methylated and ethylated derivatives (**9** and **10**) was observed.

In brief, a substituent at the methylene bridge seems to be disadvantageous to the activity of benzindoloazecines. However, it should be kept in mind that the compounds were obtained as a mixture of two different enantiomers, and it could be contemplated that the separate enantiomers would display different affinities.

A 7-methylbenzindoloazecine, substituted at C-15 with a methoxy group, could not be obtained; reduction of the precursory cyano derivative **14** failed to produce the desired azecine. (**Chapter 4.2.2**)



**Figure 5.9:** LE300 derivatives substituted at the methylene bridge.

The cyano derivative was also tested for its affinities for the dopamine receptors, where it was completely inactive. This could be anticipated, since the basicity of the alicyclic nitrogen

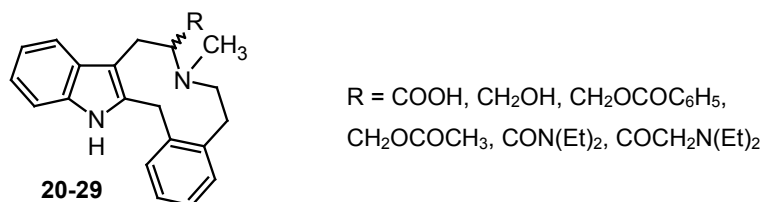
is completely demolished; and hence a crucial pharmacophoric group, namely the protonated central nitrogen interacting with Asp 3.32 in the receptor binding pocket, is lost.

### 5.3.3. LE300 substituted at position 8

The structure of LE300 was further modified by preparing derivatives substituted at position 8 with various groups. One of the main goals was to obtain the (8*R*)- and (8*S*)- enantiomers separately to study the effect of the stereochemistry on the affinities for the dopamine receptors. Many of the obtained derivatives were however found to be enantiomerically impure and only a few could be obtained in an enantiopure form. In the following, the obtained derivatives will be discussed separately according to their enantiopurity.

#### 5.3.3.1. Extensively racemized 8-substituted LE300 derivatives

As described in **chapter 4.3** and **paper 4**, the indolobenzazecines depicted in **figure 5.10** were all obtained in an almost completely racemized form, although the synthetic route was started from L- and D-tryptophan separately. Evidence thereof was gathered by determining the optical rotation and investigating the enantiomeric purity of some derivatives by chiral HPLC using Teicoplanin T as chiral stationary phase (conducted by Dr. Hefnawy, King Saud University). Conclusive was also the comparison of both the optical activity and the measured affinities ( $K_i$  values) of the herein obtained 8-hydroxymethyl derivatives and of those obtained through an alternative synthetic route (**paper 4**). While the former displayed almost no difference in the affinities between the “alleged” *R*- and *S*-enantiomers, a remarkable difference was observed between the affinities determined for the “confirmed” *R*- and *S*-enantiomers, obtained through the alternative synthetic route (**paper 4**).

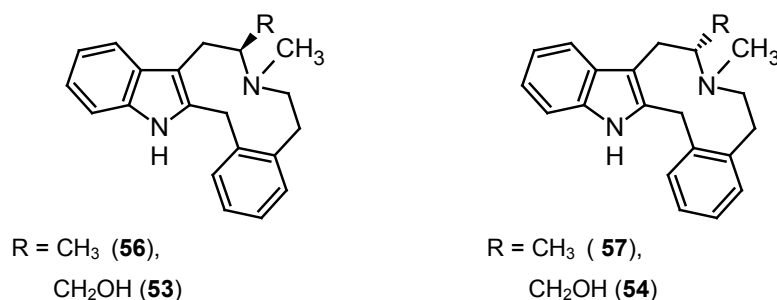


**Figure 5.10:** Racemized 8-substituted LE300 derivatives. (Cf. **chapter 4.3.1**)

Since the prepared compounds have been proved to be a mixture of two enantiomers, little could be deduced regarding the effect of the nature of the substituents on the affinities for the dopamine receptors. One highly interesting finding was however that the partially racemized amino acid derivative (**20** and **21**) showed a substantial D<sub>5</sub>/D<sub>1</sub> selectivity. This is highly attractive, since there are seldom compounds which are able to differentiate between these

two receptor subtypes, a fact which has impeded the pharmacological investigation of the roles of both receptors<sup>120</sup>.

### 5.3.3.2. Enantiopure 8-substituted LE300 derivatives



**Figure 5.11:** Enantiopure 8-methyl and 8-hydroxymethyl LE300 derivatives.

Starting from D- and L-tryptophan as well as from racemic  $\alpha$ -methyltryptamine, enantiomerically pure (8*R*)- and (8*S*)-hydroxymethyl- in addition to (8*R*)- and (8*S*)-methyl LE300 were successfully obtained (**paper 4**). (**Figure 5.11**)

First for clarification reasons, it has to be mentioned that the assignment of the configuration differs from the hydroxymethyl to the methyl derivatives. This is due to the change in the priorities of the groups involved at the chiral centre; a hydroxymethyl group has a different priority than a methyl group. For instance, in the compounds where the 8-substituent points behind the plane (dashed bond, **figure 5.11**) the methylated derivative **57** is assigned an *S*-configuration while the hydroxymethyl one **54** has an *R*-configuration.

The results obtained from the radioligand binding experiments were quite interesting; the separate enantiomers showed completely different affinities at the dopamine receptors.

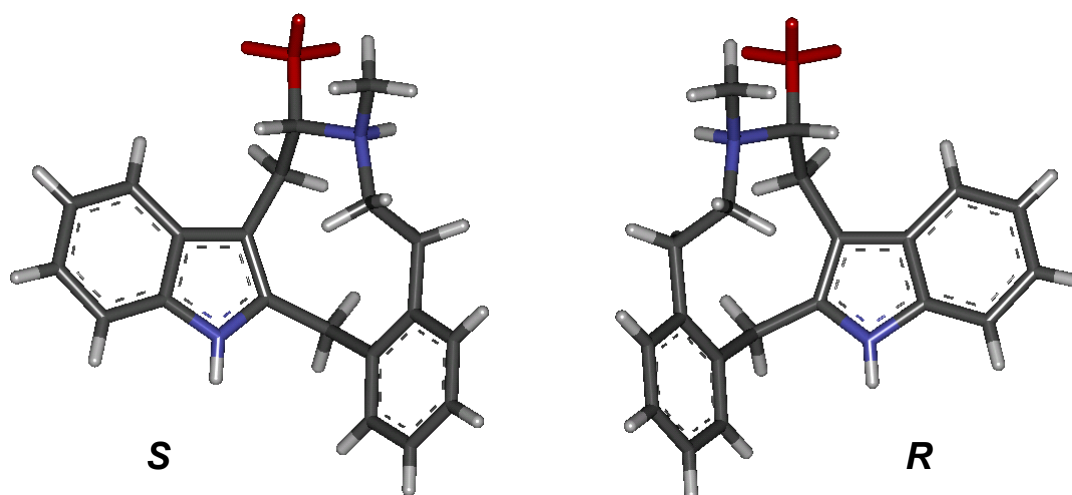
The *S*-enantiomer of 8-methylated LE300 (compound **57**, **table 5.1**), was almost as affine as the lead LE300, indicating that a methyl group at that position is well tolerated. Meanwhile, the *R*-enantiomer (compound **56**, **table 5.1**) was at least 100-fold less active than the *S*-enantiomer and was practically inactive at the  $D_2$ -like receptors ( $K_i > 10000$  nM).

A similar but less pronounced difference was observed between the *R*- and *S*- enantiomers of 8-hydroxymethyl LE300 (**table 5.1**, compounds **54** and **53**, respectively). The *R*-enantiomer was more active than the *S*-enantiomer, however only a tenfold difference in affinities was found. While the *R*- enantiomer of 8-hydroxymethyl LE300 was relatively less active than its 8-methylated counterpart (the *S*-enantiomer), the (8*S*)-hydroxymethyl derivative was more active than the (8*R*)-methylated one.

In an attempt to explain the discrepancy in the affinities of the *S*- and *R*- enantiomers of 8-methyl LE300, preliminary modelling studies were carried out. (**Chapter 4.3.2**) In the crystal

structure of LE300 HCl, two different 'enantiomers' were previously found, which has been attributed to a sort of axial isomerism, originating at the methylene group connecting both aromatic moieties. The benzindoloazecine scaffold exists in a cage-like structure, in one isomer the indole ring is on the right side of the bend structure and in the other on the left one. Which of the isomers is more active at the receptor binding site has not yet been discerned.

Introduction of a methyl group at position 8 most likely forces the structure into one of the axial isomeric forms. (Cf. **chapter 4.3.2**) Based on internal energy calculation, it could be assumed that in the *S*-enantiomer the indole ring (and the 8-methyl group) is on the left side of the bend azecine ring, and vice versa in the *R*-enantiomer. Consequently, the decreased affinities of the *R*-enantiomer could have two possible explanations: First, the position of the indole ring in the *R*-enantiomer with respect to the cage-like structure is less active at the receptor binding site. Second, the additional methyl group in the *R*-enantiomer is sterically hindered at the binding pocket, in contrast to its position in the *S*-enantiomer. (**Figure 5.12**)

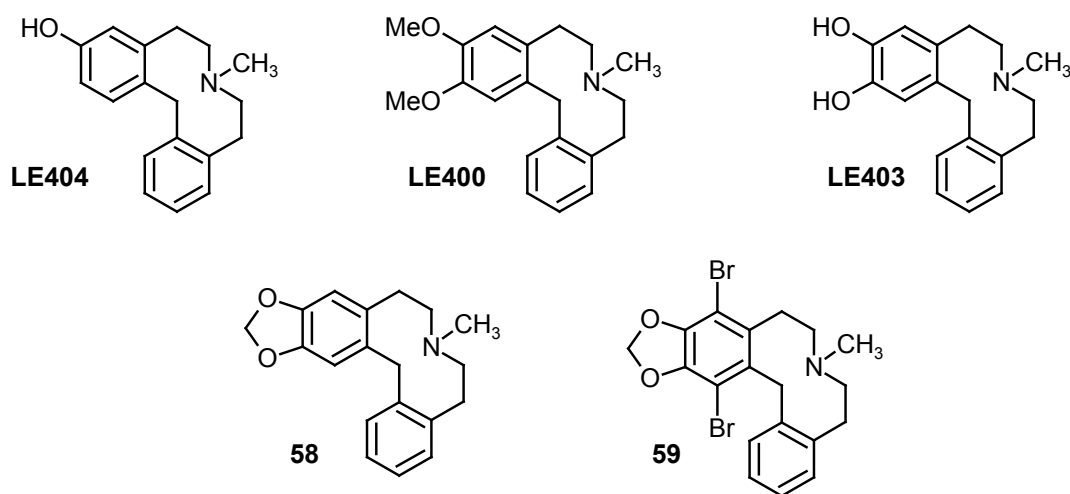


**Figure 5.12:** According to calculations of potential energy, the *S*- and *R*-enantiomers would adopt the herein depicted axial isomeric forms (or a slightly deviated one).

While trying to understand the reason behind the discrepancy in the affinities of the enantiomers, focus was mainly set on the 8-methylbenzindoloazecines. The 8-hydroxymethyl derivatives showed similar results, the *R*-enantiomer **54** (congener of the active (8*S*)-methyl derivative **57**) was more active than the *S*-enantiomer **53**. That the hydroxymethyl group also forces the scaffold into one of the axial isomeric forms could well be expected. The higher affinity of the 8*S*-hydroxymethyl derivative with respect to its 8-methyl congener (the *R*-enantiomer), might be attributed to an additional interaction of the hydroxyl group at that position with the receptor, which is not possible in case of a methyl substituent.

#### 5.4. Methylenedioxydibenzazecine derivative

Thus far, all highly affine dibenzazecines were phenolic in nature<sup>109</sup>. Most of the previously prepared dibenzazecines bear either a hydroxyl or methoxy group at the benzene ring, where a 3-hydroxyl substituent was most favored in terms of affinities. 2,3-Dihydroxy-(LE403) or 2,3-dimethoxydibenzazecine (LE400) showed a strongly attenuated activity<sup>106</sup>, which was unexpected particularly with the 2,3-dihydroxy derivative, as it bears the highest structural similarity to the natural ligand dopamine. (**Figure 5.13**)



**Figure 5.13:** Novel dibenzazecine derivative bearing a methylenedioxy moiety instead of the conventional hydroxyl or methoxy group.

The high affinities of the phenolic dibenzazecines are however coupled with some drawbacks; LE404 for instance was found to have a low oral bioavailability (**paper 3**) presumably due to the fast biotransformation of the phenolic group.

To further assess the effect of the substitution pattern on the affinities of the compounds and simultaneously evade another phenolic substituent, a dibenzazecine derivative bearing a methylenedioxy substituent at the 2,3-position (**figure 5.13**) was prepared and examined for its affinities for the dopamine receptor subtypes D<sub>1</sub>-D<sub>5</sub>. This azecine was further dibrominated to examine the impact of the additional bromine atoms on the affinities.

The dibenzazecine derivative **58** was found to be a highly active dopamine receptor antagonist, showing nanomolar affinities comparable with those of the lead LE404. Moreover, it was superior to previously synthesized 1- and 2-monohydroxylated and the 1-, 2- and 3-monomethoxylated dibenzazecine derivatives in terms of affinities. Compared with 2,3-dihydroxy-(LE403) and the 2,3-dimethoxydibenzazecine (LE400) it displayed a 100 to 1000-fold improvement in affinities. This could be explained by the fact that the rotation of the

methylenedioxy moiety is restrained and thus it occupies less space than that required for dimethoxy or even methoxy substituents.

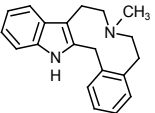
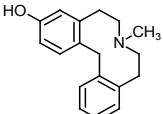
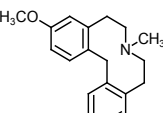
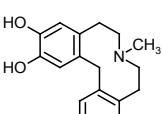
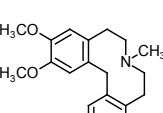
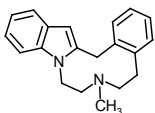
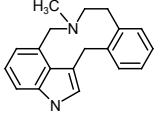
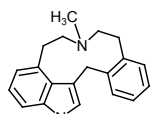
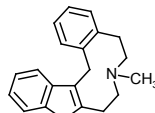
The dibrominated derivative **59** generally exhibited lower affinities than the unbrominated one, however the affinities were still considerably high and lied in the nanomolar range, with the exception of the D<sub>4</sub> receptors where the affinities were significantly attenuated.

### 5.5. Antipsychotic potential

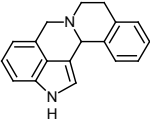
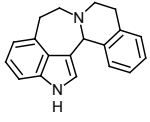
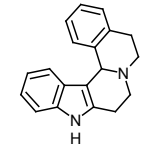
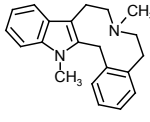
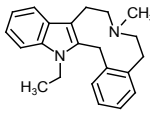
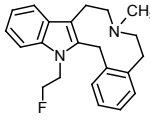
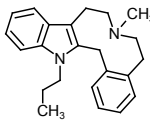
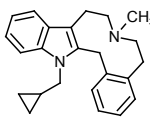
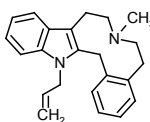
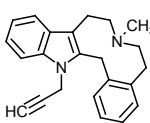
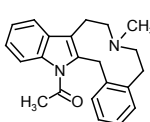
Since the indole-*N* methylated LE300 derivative **42** displayed remarkably high affinities for all dopamine receptors together with a low cytotoxicity (**paper 2**), it was selected among other compounds to test its antipsychotic potential *in vivo*. For this purpose, the ability of the compound to inhibit conditioned avoidance response (CAR) in a pole jump model was tested as a measure for its effectiveness against positive symptoms. Moreover, the tendency to induce catalepsy (bar and cross leg models) was studied as an indication for its potential to cause EPS.

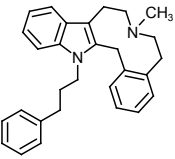
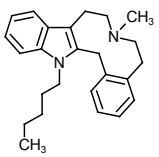
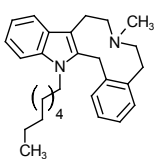
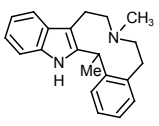
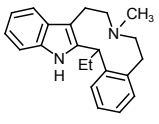
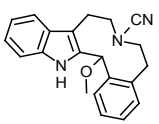
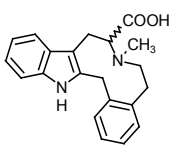
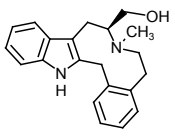
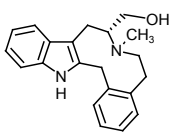
Results showed that the indole-*N* methylated LE300 (**42**) has a high potential to inhibit CAR (ED<sub>50</sub> = 0.7 mg/kg) comparable with that of the antipsychotic drug risperidone. In addition, a low tendency to induce catalepsy was observed; cataleptic behavior occurred only at doses much higher than those required for effective inhibition of CAR. It can be concluded that compound **42** has a high therapeutic range; the CAT/CAR ratio ranged between 8.7 and 11.7, which is better than that found for the antipsychotics haloperidol and risperidone. Among the tested azecine derivatives, it showed the second best results after the dibenzazecine LE404. Meanwhile it was superior to LE404 in the preliminary oral bioavailability assessment. These results render the indole-*N* methylated LE300 derivative **42** attractive as potential antipsychotic. (**Paper 3**)

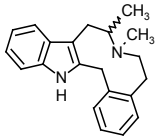
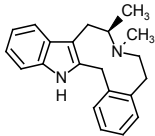
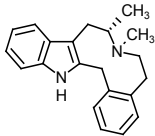
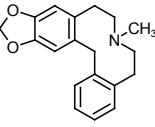
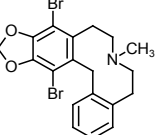
**Table 5.1:** Affinities ( $K_i$ , nM) of some relevant lead compounds and the new azecines for the dopamine receptors, determined by radioligand binding experiments.

Compounds	$K_i$ , (nM)				
	D <sub>1</sub>	D <sub>2L</sub>	D <sub>3</sub>	D <sub>4.4</sub>	D <sub>5</sub>
<b>Lead compounds</b>					
<b>LE300*</b> 	1.9 ± 0.9 <sup>a</sup>	44.5 ± 15.8 <sup>a</sup>	40.3 ± 4.4 <sup>a</sup>	109 ± 39 <sup>a</sup>	7.5 ± 0.3 <sup>a</sup>
<b>LE404*</b> 	0.39 ± 0.22 <sup>b</sup>	17.5 ± 1.5 <sup>b</sup>	47.5 ± 24 <sup>c</sup>	11.3 ± 1.0 <sup>b</sup>	1.5 ± 0.5 <sup>b</sup>
<b>LE425*</b> 	28.5 ± 9.7 <sup>c</sup>	13.0 ± 9.0 <sup>c</sup>	75.7 ± 7.3 <sup>c</sup>	43.4 ± 13.3 <sup>d</sup>	54.0 ± 20 <sup>d</sup>
<b>LE403*</b> 	341 ± 41 <sup>b</sup>	>5000 <sup>b</sup>	n.d.	165 ± 12 <sup>b</sup>	1078 ± 42 <sup>b</sup>
<b>LE400*</b> 	509 ± 51	>5000	n.d.	2514 ± 101	2610 ± 120
<b>Modulation of the annulation pattern of LE300</b>					
<b>37*</b> 	210 <sup>e</sup>	13.2 <sup>e</sup>	n.d.	n.d.	n.d.
<b>1</b> 	>10000	>10000	>10000	>10000	>10000
<b>2</b> 	4.2 ± 0.5	4.0 ± 0.5	42.9 ± 8.5	39.1 ± 9.6	2.5 ± 0.7
<b>38</b> 	28.5 ± 7.2	55.1 ± 5.4	513 ± 217	225 ± 50	10.3 ± 1.5



Compounds		$K_i$ (nM)				
		D <sub>1</sub>	D <sub>2L</sub>	D <sub>3</sub>	D <sub>4,4</sub>	D <sub>5</sub>
39		>10000	>10000	>10000	>10000	>10000
40		1836 ± 658	4458 ± 1192	>10000	>10000	1525 ± 206
41		2101 ± 703	1476 ± 28.5	>10000	>10000	>10000
<b><u>N14-substituted LE300</u></b>						
42		2.0 ± 0.5	8.2 ± 5.8	7.2 ± 2.8	13.2 ± 3	0.5 ± 0.1
43		3.2 ± 0.8	51.7 ± 12.4	181 ± 66	65.5 ± 28.9	7.5 ± 1.5
44		12.6 ± 3.6	54 ± 5	119 ± 24	11.9	3.8 ± 0.8
45		22.3 ± 7.3	197 ± 5.5	307 ± 15	84 ± 11	9.8 ± 2.6
46		14.3 ± 0.2	241 ± 17	305 ± 68	39 ± 24	57 ± 40
47		27 ± 15	28.9 ± 9.3	99.3 ± 15	9.1 ± 3.6	5.5 ± 2
48		6.9 ± 0.2	128 ± 45	411 ± 81	36.5	1.2 ± 0.3
49		22.7 ± 9.7	14.2 ± 1.3	158 ± 46	126 ± 0	9.9 ± 1.2

Compounds	$K_i$ , (nM)				
	D <sub>1</sub>	D <sub>2L</sub>	D <sub>3</sub>	D <sub>4.4</sub>	D <sub>5</sub>
<b>50</b> 	116 ± 30	356 ± 16	810 ± 169	1327 ± 535	25.6 ± 5.3
<b>51</b> 	23.2 ± 12	773 ± 160	390 ± 67.5	455 ± 8.0	261 ± 65
<b>52</b> 	184 ± 60	3383 ± 694	1760 ± 532	1414 ± 531	77.3 ± 3
<b>LE300 bearing substituents between the aromatic rings</b>					
<b>9*</b> 	49 ± 7	200 ± 32	>10000	533 ± 45	64 ± 28
<b>10</b> 	116 ± 66	725 ± 102	>10000	298	21.7 ± 3
<b>14</b> 	>10000	>10000	n.d.	>10000	>10000
<b>8- substituted LE300</b>					
<b>20</b> 	>10000	>10000	>10000	>10000	657 ± 163
<b>53</b> 	114 ± 9	2923 ± 442	4676 ± 1593	4861 ± 803	91.4 ± 8.5
<b>54</b> 	10.3 ± 1.4	268 ± 56	681 ± 68	408 ± 31	11.4 ± 2.3

Compounds	$K_i$ (nM)				
	D <sub>1</sub>	D <sub>2L</sub>	D <sub>3</sub>	D <sub>4,4</sub>	D <sub>5</sub>
<b>55</b> 	32.4 ± 13.3	526 ± 69	385 ± 3.5		16.9 ± 6.5
<b>56</b> 	640 ± 119	>10000	>10000	>10000	389 ± 0.8
<b>57</b> 	6.1 ± 2.2	85.3 ± 15.2	304 ± 1	64.1 ± 19.3	4.4 ± 2.5
<hr/>					
<b><u>Methylenedioxy-dibenzazecine derivatives</u></b>					
<b>58</b> 	3.6 ± 1	5.5 ± 1.2	20.6 ± 5.5	46.0 ± 9.9	0.5 ± 0.01
<b>59</b> 	35.0 ± 14.5	30.0 ± 19.6	38.9 ± 1.1	2760 ± 552	4.7 ± 1.9

\* Previously reported. <sup>a</sup> Values taken from reference <sup>108</sup>. <sup>b</sup> Values taken from reference <sup>106</sup>. <sup>c</sup> Values taken from reference <sup>109</sup>. <sup>d</sup> Values taken from reference <sup>110</sup>. <sup>e</sup> Values taken from reference <sup>119</sup>.

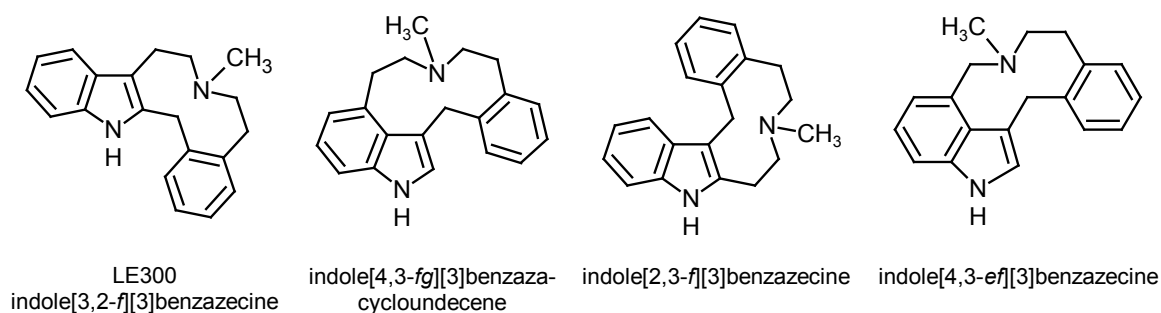
## 6. Conclusion

Benzindoloazecines and dibenzazecines compose a new class of dopamine receptor antagonists, which has attracted attention due to its structural novelty, their high affinities for dopamine receptors and their selectivity for the D<sub>1</sub>-like receptors. Extensive SAR studies have been conducted on these azecine-type dopamine receptor antagonists by manipulating the structure of both lead compounds, the benzindoloazecine LE300 and the dibenzazecine LE404. Focal point of the presented work was to further modify the structure of both leads while basically maintaining these structural features, previously established to be imperative for high activity. Work has mainly focused on modifying the structure of LE300 by changing its annulation pattern or introducing substituents at various positions of its tetracyclic ring system. For this purpose, a novel modified synthetic procedure was applied for a more efficient synthesis of several precursory quinolizines. Moreover, the structure of the lead dibenzazecine derivative LE404 was modified by replacing the 3-hydroxyl group by a methylenedioxy moiety.

The goal was to further develop SAR studies on this novel class of dopamine receptor antagonist with the aspiration of attaining compounds with improved affinities, higher subtype selectivity or better pharmacokinetics. A total of 29 novel azecine derivatives have been prepared, their affinities at the dopamine receptors were tested by radioligand ligand binding assay and their functionality by an established Ca<sup>2+</sup> assay.

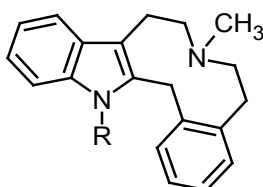
Based on the results obtained in this work, the following conclusions can be made:

- The herein introduced alterations in the annulation pattern of LE300, namely by converting its indolo[3,2-*f*][3]benzazecine scaffold to an indolo[4,3-*fg*][3]benzazacycloundecene and indolo[2,3-*f*][3]benzazecine one, did not lead to pronounced effects on the affinities, suggesting that the distances between the different pharmacophores and the flexibility of this ring system rather than its annulation pattern have the main effect on the affinities.



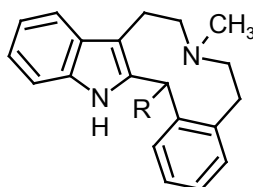
This is supported by the lack of activity of the more rigid indolo[4,3-*ef*][3]benzazecine derivative, which encompasses shorter distances between the pharmacophoric groups, specifically between the indole ring and the central nitrogen.

- Substitution of the indole-*NH* by various residues led to varying alterations in the affinities and selectivity profile. A methyl group increased the affinities for all dopamine receptors, except for D<sub>1</sub>. An ethyl residue was well tolerated and did not significantly affect the affinities when compared with the lead LE300, while longer and bulkier residues mostly led to a proportionate decrease in affinities. The relative affinities for the D<sub>4</sub> receptor were generally increased; this was most remarkable with the allylated derivative, which should the highest affinity for the D<sub>4</sub> receptor.



Worth mentioning is that the indole-*N* methylated LE300 showed a promising antipsychotic effect *in vivo*, with a low potential to cause EPS. Moreover, primary results infer that it possesses a good oral bioavailability, better than the lead compound LE404.

- Small substituents (methyl and ethyl) at the methylene bridge connecting both aromatic moieties were much less tolerated, especially at the D<sub>3</sub> receptor. A clear-cut conclusion on the effect of the substituents at that position on the affinities could however not be made, since the derivatives were obtained as racemates; the effect of the configuration of the substituent on the affinities could hence not be determined.

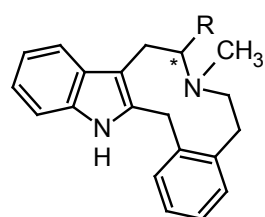


- The effect of the substituent at the 8-position of the benzindoloazecine ring depended on the nature of the substituent and more pronouncedly on its configuration. Both enantiopure derivatives, where the substituents point below the plane, namely (8*S*)-methyl and (8*R*)-hydroxymethyl LE300, showed similar or slightly decreased affinities

than LE300. The derivatives bearing the opposite configuration, i.e. (8*R*)-methyl and (8*S*)-hydroxymethyl LE300, exhibited a substantial decrease in affinities; almost a 100-fold decrease for the methyl- and a 10-fold for the hydroxymethyl derivative. The D<sub>1</sub>-selectivity profile was retained with all derivatives.

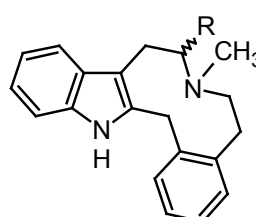
Preliminary investigations indicate that the additional substituent at the 8-position forces the structure into only one of the axial isomers, compared to the two isomers 'enantiomers' found in LE300. Hence, the difference in the affinity of the enantiomers could arise either from the spatial orientation of the 8-methyl group or the orientation of the existing axial isomer (indole to the right or to the left of the bent azecine structure).

The effect of other substituents at position 8 could not be accurately assessed, since the various derivatives were obtained in an enantiomerically impure form. However, a highly attractive derivative was obtained, namely that bearing a carboxylic acid group at position 8, as it showed practically no affinities for all dopamine receptors, except for D<sub>5</sub> and thus a rarely encountered D<sub>5</sub> selectivity.



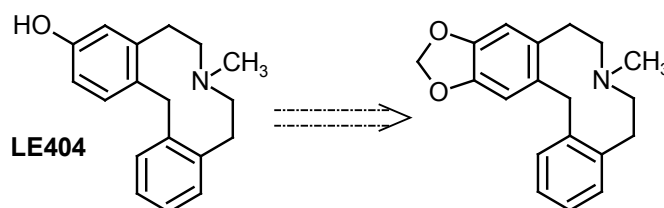
\* *R* and *S*

*R* = CH<sub>3</sub>, CH<sub>2</sub>OH



*R* = COOH, CH<sub>2</sub>OCOC<sub>6</sub>H<sub>5</sub>, CH<sub>2</sub>OCOCH<sub>3</sub>,  
CON(Et)<sub>2</sub>, COCH<sub>2</sub>N(Et)<sub>2</sub>

- The first highly active nonphenolic analogue of LE404 was prepared, namely by substitution of the 2,3-position with a methylenedioxy moiety. This derivative showed nanomolar affinities for all dopamine receptors, the selectivity for the D<sub>1</sub>-like receptors was however lost. This indicates that the rather pharmacokinetically unflavored phenolic group is not imperative for high affinities, and could be replaced with a methylenedioxy moiety; which is expected to be of better pharmacokinetics and chemical stability than previously prepared highly affine phenolic benzazecines, such as the lead compound LE404



## 7. Zusammenfassung

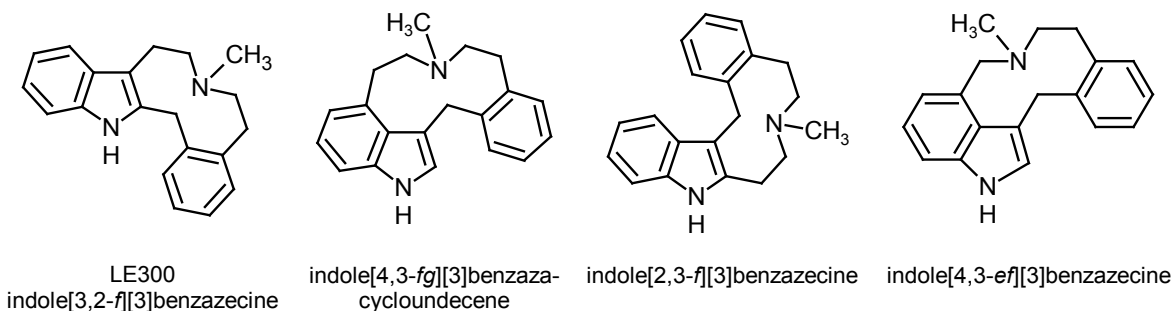
Azecine sind eine neuartige Klasse von Dopaminrezeptor-Antagonisten, gekennzeichnet durch ihre nanomolare Affinitäten an den Dopaminrezeptoren und vor allem ihre Selektivität für die Rezeptoren der D<sub>1</sub>-Familie. Strukturwirkungsbeziehungen dieser Verbindungsklasse wurde umfassend erforscht. Die Strategie der vorliegenden Arbeit war die Strukturen der Leitverbindungen LE300 und LE404 so zu modifizieren, dass die erforderlichen strukturellen Merkmale weitgehend erhalten werden sollten. Das Benzindoloazecin LE300 wurde vielfältig modifiziert; z.B. durch Veränderung des Annullierungsmusters vom Ringgerüst oder Einfügen eines Substituenten an verschiedenen Stellen. Außerdem wurde die phenolische Gruppe der Leitstruktur LE404 vom Dibenzazecin-Typ durch eine Methylendioxygruppe ersetzt.

Das Ziel dieser Arbeit war die Strukturwirkungsbeziehungen dieser neuartigen Klasse von Dopaminrezeptor-Antagonisten weiter zu entwickeln, um Derivate mit höheren Affinitäten und besser ausgeprägten Subtypselektivität zu erreichen.

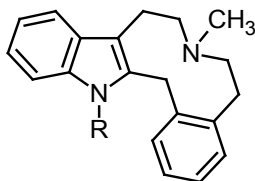
Insgesamt wurden 29 Zielverbindungen synthetisiert und die Affinitäten an den verschiedenen Dopaminrezeptoren (human) durch Radioligandbindungsstudien ermittelt. Weiterhin wurde die Funktionalität der Verbindungen (Agonisten oder Antagonisten) mit Hilfe eines Calcium Assays bestimmt.

Folgende Strukturwirkungsbeziehungen konnten erkannt werden:

- Die Änderung des Annullierungsmusters von LE300 hatte keinen signifikanten Einfluss auf die Affinitäten; die Indolo[4,3-*fg*][3]benzazacycloundecen und Indolo[2,3-*f*][3]benzazecin Derivate zeigten ähnliche oder etwas niedrigere Affinitäten als die Leitverbindung LE300. Hingegen führte eine Verkürzung der Distanzen zwischen den Pharmakophoren, und die damit verbundene Verringerung der Flexibilität, zu einem kompletten Affinitätsverlust wie es sich beim Indol[4,3-*ef*][3]benzazecin Derivat zeigte.

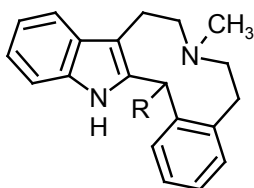


- Die Substitution des Indol-*NH* vom Benzindoloazecin LE300 mit verschiedenen Gruppen hatte unterschiedliche Auswirkungen auf die Affinitäten. Die Einführung einer Methylgruppe am Indol-*NH* führte zu einer signifikanten Affinitätssteigerung an allen Dopaminrezeptoren außer am D<sub>1</sub>-Rezeptor. Eine Ethylgruppe wurde toleriert, wobei längere Ketten verminderte Affinitäten zeigten. Eine Affinitätssteigerung an den D<sub>4</sub>-Rezeptoren konnte bei allen Derivaten festgestellt werden, insbesondere beim Allylderivat.



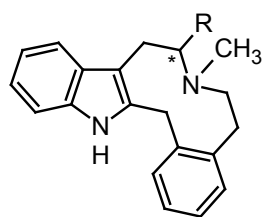
In den Tierversuchen an Ratten zeigte die methylierte LE300 Verbindung eine hohe neuroleptische Potenz hinsichtlich der Aufhebung der Positivsymptomatik und zeigten dazu eine geringere Tendenz extrapyramidale Nebenwirkungen hervorzurufen. Ausserdem konnte bei der Methylverbindung eine gute orale Bioverfügbarkeit festgestellt werden.

- Kleine Substituenten an der Methylengruppe zwischen beiden Aromaten führten im Allgemeinen zu Affinitätsverlusten, besonders am D<sub>3</sub> Rezeptor. Der Einfluss der Substituenten an dieser Stelle konnte aber nicht gründlicher beschrieben werden, da die Derivate als Razemate erhalten wurden, und der Einfluß der Konfiguration von Substituenten auf die Affinitäten nicht bestimmt werden konnten.



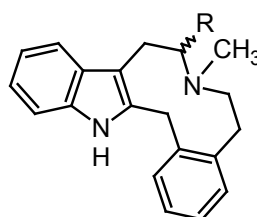
- Die Einführung verschiedener Substituenten in der 8-Position des Benzindoloazecin-Ringgerüsts hatte unterschiedliche Auswirkungen, die von der Art und Konfiguration des Substituenten abhängig waren. Enantiomerenreine Verbindungen, bei dem der Substituent nach hinten zeigte, nämlich (8*S*)-Methyl und (8*R*)-Hydroxymethyl LE300, zeigten ähnliche oder etwas niedrigere Affinitäten als die Leitverbindung LE300. Hingegen zeigten (8*R*)-Methyl und (8*S*)-Hydroxymethyl LE300 eine 100-fache bzw. 10-fache Affinitätssenkung.





\* R and S

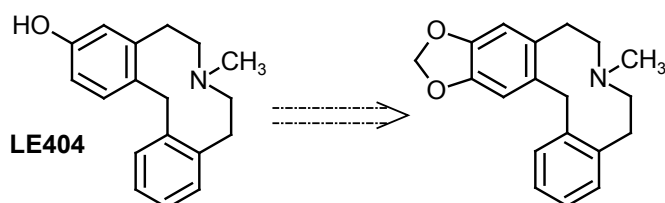
R = CH<sub>3</sub>, CH<sub>2</sub>OH



R = COOH, CH<sub>2</sub>OCOC<sub>6</sub>H<sub>5</sub>, CH<sub>2</sub>OCOCH<sub>3</sub>,  
CON(Et)<sub>2</sub>, COCH<sub>2</sub>N(Et)<sub>2</sub>

Der Einfluss anderer Substituenten an dieser Stelle konnte nicht genau bestimmt werden, da die Derivate in einer racemisierten Form erhalten wurden. Interessant war jedoch, dass Aminosäurederivat (substituiert mit einer Carbonsäure an der 8-Position.), praktisch keine Affinitäten an allen Dopaminrezeptoren zeigte außer am D<sub>5</sub>-rezeptor, und damit eine seltene D<sub>5</sub>-Selektivität darstellt.

- Eine hochaffine nicht-phenolische Dibenzazecinverbindung wurde erhalten, indem die phenolische OH-Gruppe mit einer Methylendioxygruppe an der 2,3-position ersetzt wurde. Die neue Verbindung zeigte nanomolare Affinitäten, vergleichbar mit der Leitsubstanz LE404, aber auch einen Verlust der D<sub>1</sub> Selektivität. Es wird erwartet dass die Verbindung eine bessere Pharmakokinetik, unter anderem bessere orale Bioverfügbarkeit, als das phenolische LE404 zeigt.



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## List of Abbreviations

ADHD	Attention deficit hyperactivity disorder
AMPA	$\alpha$ -Amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid
Asp	Aspartic acid
c-AMP	Cyclic adenosine monophosphate
CAR	Conditioned avoidance response
CAT	Catalepsy
Cf.	Compare, see
CNS	Central nervous system
DARPP32	Dopamine- and c-AMP-regulated neuronal phosphoprotein 32
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EPS	Extrapyramidal symptoms
Et	Ethyl
GABA	$\gamma$ -Aminobutyric acid
GPCR	G-protein coupled receptors
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
KO	Knockout
Me	Methyl
MeOH	Methanol
NMDA	<i>N</i> -Methyl-D-aspartic acid
NMR	Nuclear magnetic resonance
OMe	Methoxide
PFC	Prefrontal cortex
PKA	Protein kinase A
SAR	Structure activity relationship
Ser	Serine
THF	Tetrahydrofuran
THPB	Tetrahydroprotoberberine
TM	Transmembrane domain
VTA	Ventral tegmental area

## Curriculum Vitae

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## List of publications

### Manuscripts

Novel benzothiazole-2-carbamates as potential anthelmintic agents.

Omar, A.-M. M. E., Ahmed, I. C., El-Sayed, O. A., Youssef, A. M., Hassan, D. A. and El-Ashmawy, I.

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Residues at the Indole-NH of LE300 Modulate Affinities and Selectivities for Dopamine Receptors.

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*Arch. Pharm. (Weinheim)* **2011**, 344, 28-36.

A Novel Nonphenolic Dibenzazecine Derivative with Nanomolar Affinities for Dopamine Receptors

Robaa, D.; Abulazm, S. E.; Lehmann, J.; and Enzensperger, C.

*Chemistry and Biodiversity*; accepted.

Synthesis and Dopamine Receptor Affinities of Racemic and Enantiopure Indolo[3,2-*f*][3]benzazecine Derivatives.

Robaa, D.; Enzensperger, C.; Hefnawy, M.; El-Subbagh, H.; Abulazm, S. E.; and Lehmann, J.

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Molecular Combination of the Dopamine and Serotonin Scaffolds Yields Novel Antipsychotic Drug Candidates – Characterization by *in vivo* Experiments.

Schulze, M.; Siol, O.; Robaa, D.; Mueller, F.; Fleck, C.; and Lehmann J.

*Arzneimittelforschung*; manuscript submitted.

### Posters

**Novel and facile procedure for the synthesis of different quinolizines as precursors of azecine-type dopamine receptor ligands**, Robert Otto, Dina Robaa, Christoph Enzensperger and Jochen Lehmann, Poster DPHG-Jahrestagung 2009.

**M14-Substitution of the dopamine antagonist LE300 modulates affinities and selectivities for dopamine D<sub>1</sub> - D<sub>5</sub> receptors**, Dina Robaa, Robert Kretschmer, Christoph Enzensperger, Shams EIDin AbulAzm, ElSayed ElKhawass and Jochen Lehmann, Poster DPHG-Jahrestagung 2009.

## **Selbstständigkeitserklärung**

Hiermit erkläre ich, dass mir die geltende Promotionsordnung der Fakultät bekannt ist.

Die vorliegende Arbeit habe ich selbstständig und nur unter Verwendung der angegebenen Hilfsmittel und Literatur angefertigt.

Ich habe weder die Hilfe eines Promotionsberaters in Anspruch genommen, noch unmittelbar oder mittelbar geldwerte Leistungen im Zusammenhang mit dem Inhalt meiner Dissertation an Dritte erbracht.

Die vorliegende Dissertation habe ich ausschließlich an der Friedrich-Schiller-Universität als Prüfungsarbeit eingereicht.

Jena, im Februar 2011

## **Acknowledgement**

This work arose out of three years of work that has been done since I have come to Jena in February 2008. By that time I have worked and met with many, whose work-related and personal contribution made research work rewarding and this period as a whole memorable. It is my pleasure to convey my utmost gratitude to them all.

First and foremost, I would like to dearly thank my supervisor; Prof. Dr. Jochen Lehmann. I am profoundly grateful to him for giving me the chance to join his research group and for allowing me to finish my work here. His advice and support has enriched the research work throughout, while his kind, relaxed and optimistic way has constantly served for a pleasant and enjoyable atmosphere.

My deepest thanks are extended to my Egyptian mentor; Prof. Dr. Shams Eldin Abulazm, for his support, for him continuously checking up on how I am doing and for always backing me up.

I am especially grateful to Dr. Christoph Enzensperger, whose clever, elegant sometimes even “wild” ideas have contributed immensely to the research work. His forthcoming and outgoing way has allowed me to quickly find my way into the research project and rendered our conversations (both work-related and unrelated) enjoyable.

All through I have been blessed with a helpful and cheerful group of colleagues at the Philosophenweg 14, on whose assistance I could always rely on. I would like to heartily thank all of them.

In particular I would like to thank my dearest friends Claudia Lengfelder and Robert Otto, for always lending me a listening ear and for their constant support, especially with everyday problems, which, given my ignorance of many German rules, has been of utmost value. I would like to thank them for the great times in the lab and the office/coffee room and for putting up with me through the tougher times.

I am very grateful to all technicians; Katrin Fischer and Monika Listing, to whom I repeatedly turned to for technical questions and Bärbel Schmalwasser, Heidi Traber and Petra Wiecha for their assistance in the pharmacological assays.

I would also like to acknowledge the financial support by the Egyptian High school ministry, which I received for the first two years of my work.

My utmost gratitude is conveyed to my family. My parents; Layla and Aly, for their care and constant encouragement, for supporting me in all my pursuits for everything. My sisters; May and Ola, my cornerstones in the last couple of years, for always having them to turn to and whose presence in Germany has made this period much more enjoyable. My brother; Nadeem, for being there and cheering me up even from half way across the world.